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THIAZOLIDINE AND THIAZOLINE DERIVATIVES OF 3-ARYL-3-TRIFLUOROMETHYLDIAZIRINES FOR THE PREPARATION OF FLUORESCENT OR ^{35}S-RADIOLABELED PHOTOAFFINITY PROBES

Stefan Kwiatkowski, Peter J. Crocker, Ashok J. Chavan, Nobuyuki Imai, Boyd E. Haley, and David S. Watt*

Department of Chemistry and Department of Biochemistry, University of Kentucky, Lexington, KY. 40506,

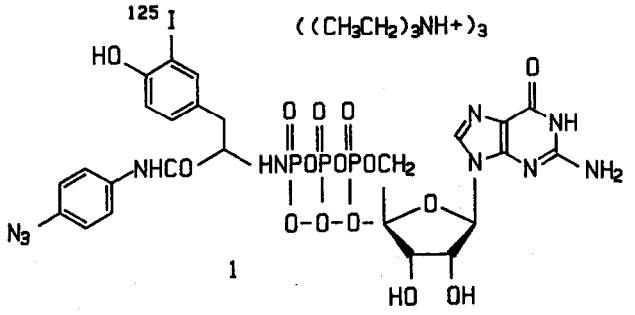
Ren-jye Ho

Department of Biochemistry, University of Miami School of Medicine, Miami, FL. 33101

Abstract. The condensation of cysteine with 3-(4-formylphenyl) or 3-(4-cyanophenyl)-3-trifluoromethyldiazirine furnished thiazolidine and thiazoline derivatives in good yield. These heterocycles provide convenient access to photoaffinity probes an ^{35}S radiolabel or a fluorescent dansyl group.

The preparation of photoaffinity cross-linking reagents for studying the interaction of secondary metabolites and their protein receptors required reagents possessing the following features: (1) an electrophilic terminus that could be linked to the natural product under study; (2) a "reporter group," such as an aryl azide or aryl diazirine that would furnish a reactive species upon photolysis; and (3) either a radiolabel of high specific activity and convenient half-life (i.e., ^{32}P, ^{35}S, ^{125}I) or a fluorophore that would permit detection of cross-linked adducts. Various combinations of photoactive groups and radiolabels have found widespread application in biochemistry,[1] and in particular, the use of probes with ^{125}I or ^{32}P radiolabels and photoactive azide groups has been popular. However, we recently encountered modest cross-linking efficiencies using radioiodinated aryl azides found in the GTP photoaffinity probe[2] 1 and a forskolin photoaffinity probe[3] 2, and we attributed these difficulties to a combination of photodeiodination[3] and modest reactivity of the intermediate dehydroazepines.[4] Consequently, we sought to incorporate an aryl diazirine[5] in place of the aryl azide group and to retain the option of using either a fluorescent tag or a photostable ^{35}S

radiolabel. We report the preparation of thiazoline and thiazolidine heterocycles that possess these features and the application of these reagents in the preparation of fluorescent or ^{35}S-radiolabeled forskolin derivatives. We are not aware of other reagents of this type that offer both versatility in terms of the "reporter group" and ease of synthesis.



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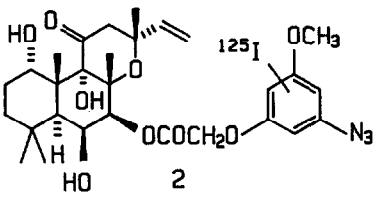

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As shown in Scheme 1, application of the Kornblum oxidation[6] to 3-(4-(bromomethyl)phenyl)-3-trifluoromethyldiazirine[7] (3) furnished the aldehyde 4. Condensation of the aldehyde 4 with R-(+)-cysteine gave the expected thiazolidine[8] 5 as a 1:1 inseparable mixture of the (2R,4R)-cis- and (2S,4R)-trans isomers possessing the same relative configuration at C-4 as in cysteine,[8b] and unlike the corresponding azides, the diazirine functionality survived exposure to thiols.[9] The reaction of 5 with dansyl chloride in pyridine gave exclusively the (2S,4R)-trans-thiazolidine 6 through a mechanism[8b] involving Schiff base formation and thiazolidine reclosure followed by reaction with dansyl chloride. Consistent with the absence of any racemization at C-4 in 6, the regioselective coupling[10] of 6 with desacetylforskolin[11] (7) provided a single diastereomer of the fluorescent forskolin derivative 8.

Conversion of the aldehyde 4 to the nitrile 9 via an oxime, and condensation of 9 with R-(+)-cysteine provided the thiazoline[12] 10 in which the configuration at C-4 was partially racemized as indicated by the ^1H NMR spectrum of the corresponding methyl ester (7:3 ratio) in the presence of $Eu(thd)_3$. The coupling of desacetylforskolin with 10 provided 11 as a separable mixture of diasteromers. The condensation of [^{35}S]-Cys with 9 and coupling to desacetylforskolin led to the [^{35}S]-labeled derivative 12. It was

of interest to find that both 8 and 12, in a fashion reminiscent of taxol and forskolin itself, enhanced the polymerization of the structural protein, tubulin, and that the fluorescent probe retained approximately 25% of the activity of forskolin to stimulate adenylate cyclase. Application of these probes to a detailed study of these problems will be reported in due course.[13]

Acknowledgement

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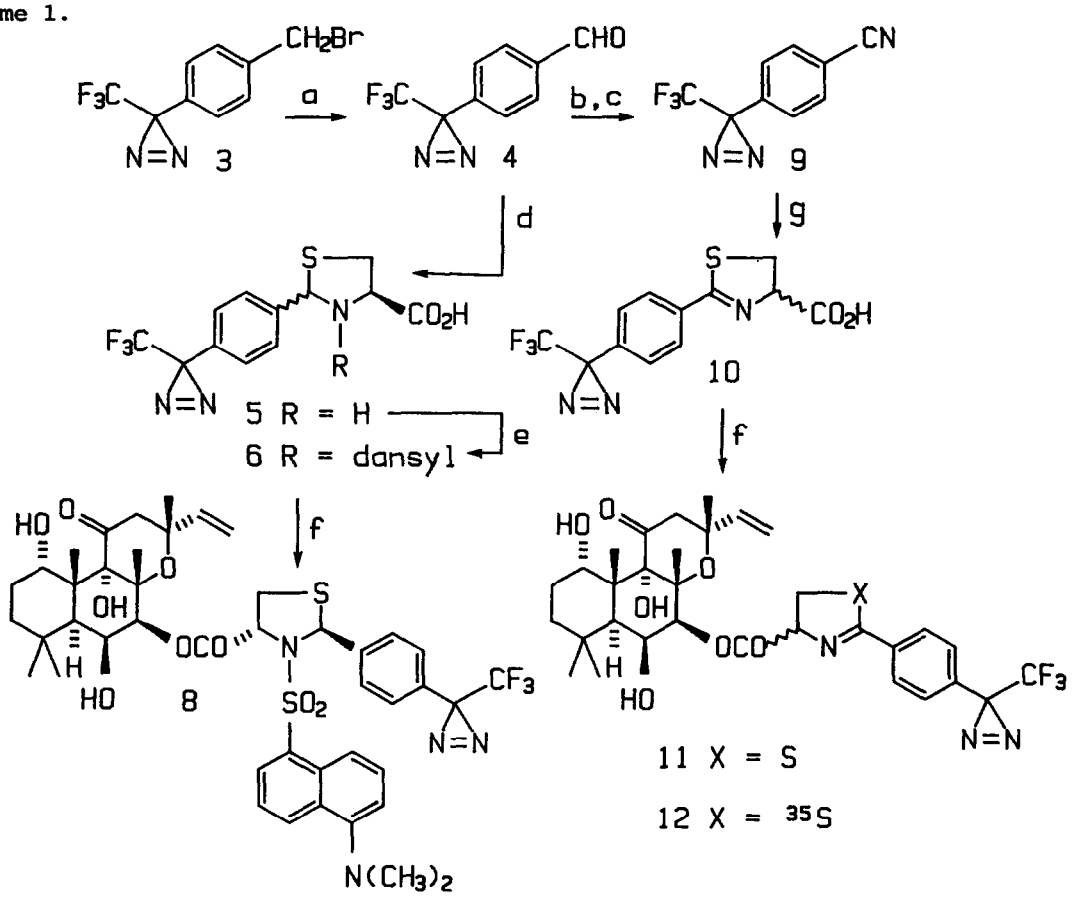
Scheme 1.



a, i-$PrNO_2$, NaOEt, EtOH, 70°C, 1 h; b, $NH_2OH \cdot HCl$, NaOH, EtOH, 50°C, 2 h; c, MsCl, Et_3N, THF, 40°C, 30 min followed by 1.0 equiv of tetramethylguanidine, 40°C, 2 h; d, L- or R-(+)-Cys, MeOH, 40°C, 4 h; e, 1.1 equiv of dansyl chloride, Py, 50°C, 24 h; f, desacetylforskolin (7), DCC, DMAP, CH_2Cl_2, 22°C, 24 h; g, L- or R-(+)-Cys, K_2CO_3, MeOH, 60°C, 18 h.

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13. All compounds were characterized by IR, NMR and exact mass or combustion analysis data. Pertinent spectral data for some of the compounds in this communication are as follows:

3-(4-Formylphenyl)-3-trifluoromethyldiazirine (4): IR (TF) 1698 (C=O), 1605 (N=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (d, J=8 Hz, 2), 7.92 (d, J=8 Hz, 2), 10.05 (s, 1, CHO); ^{13}C NMR ($CDCl_3$) δ 127.04, 130.0, 191.34; exact mass spectrum calcd for $C_9H_5F_3N_2O$-N_2 186.0292, found, 186.0287.

3-[4-(4-Carboxy-2-thiazolidinyl)phenyl]-3-trifluoromethyldiazirine (5): a 52:48 mixture of cis and trans isomers: dp 131-133°C; $[\alpha]_D$= -88.3° (c=0.725, ethanol); IR (KBr) 3100-2300 (NH_2^+), 1580 (CO_2^-); trans-isomer (2S,4R): ^1H NMR ($CDCl_3$) δ 3.45 (dd, J=6.8 and 10.2 Hz, 1, C-5 H), 3.67 (dd, J=7.0 and 10.2 Hz, 1, C-5 H), 4.44 (dd, J=6.8 and 7.0 Hz, 1, C<u>H</u>CO_2H), 6.20 (s, 1, C<u>H</u>SCH_2), 7.15 (d, J=8.0 Hz, 2, ArH), 7.69 (d, J=8.0 Hz, 2, ArH), 8.3 (br s, 2, NH, OH); cis-isomer (2R,4R): ^1H NMR ($CDCl_3$) δ 3.43 (dd, J=8.8 and 10.2 Hz, 1, C-5 H), 3.55 (dd, J=7.2 and 10.2 Hz, 1, C-5 H), 4.29 (dd, J=8.7 and 7.2 Hz, 1, C<u>H</u>CO_2H), 5.78 (s, 1, C<u>H</u>SCH_2), 7.16 (d, J=8.0 Hz, 2, ArH), 7.67 (d, J=8.0 Hz, 2, ArH), 8.3 (br s, 2, NH, OH); exact mass spectrum calcd for $C_{12}H_{10}F_3N_3O_2S$ 317.0446, found 317.0445.

3-(4-Cyanophenyl)-3-trifluoromethyldiazirine (9): IR (TF): 2240 (C≡N), 1610 (N=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (d, J=8 Hz, 2), 7.72 (d, J=8 Hz, 2); ^{13}C NMR ($CDCl_3$) δ 113.76 (C≡N), 127.14, 132.65. Anal. Calcd. for $C_9H_4F_3N_3$: C, 51.20; H, 1.91. Found: C, 51.09; H, 1.92.

3-[4-(4-Carboxy-2-thiazolinyl)phenyl]-3-trifluoromethyldiazirine (10): dp 130-132°C (from 1:1 EtOAc-hexane); IR (KBr) 1730 (C=O), 1598 (N=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.76 (d, J=9.4 Hz, 2, CH_2), 5.38 (t, J=9.4 Hz, 1, CH), 6.02 (br s, 1, CO_2H), 7.24 (d, J=8.2 Hz, 2, ArH), 7.89 (dt, J=8.8 and 2.0 Hz, 2, ArH); ^{13}C NMR ($CDCl_3$) δ 35.24; 77.96, 126.61, 128.91, 132.97, 171.65, 173.03. Anal. Calcd. for $C_{12}H_8F_3N_3O_2S$: C, 45.72; H, 2.56. Found: C, 45.95; H, 2.59.

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