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Research Article

Synthesis of covalent [^{14}C]-labeled diarylsulfonylurea (DASU) inhibitors of the processing and release of IL-1

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Summary

CP-452,759 and CP-470,947, two covalent [^{14}C]-labeled diarylsulfonylurea (DASU) inhibitors of the processing and release of IL-1 from human monocytes, have been synthesized. The radiosyntheses utilize [^{14}C]-labeled phosgene to prepare labeled isocyanates which are coupled with an epoxide sulfonamide to give the [^{14}C]-DASUs. These tools should allow future studies aimed at purifying and identifying the DASU binding protein involved in the inhibition of the processing and release of IL-1 from human monocytes. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: [^{14}C]phosgene; [^{14}C]–labeled covalent DASUs; IL-1 inhibitors

Introduction

The cytokine interleukin-1 (IL-1) is a key mediator in the inflammatory response.[1] Levels of this cytokine are elevated in synovial fluid isolated from inflamed joints of patients with osteoarthritis and rheumatoid arthritis.[2] Furthermore, repeated injection of IL-1 into the joints of normal rats results in chronic synovitis.[3] Aberrant production of this

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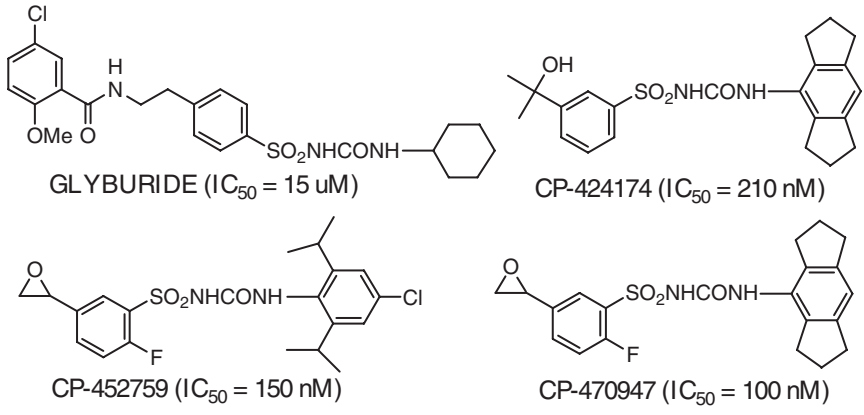


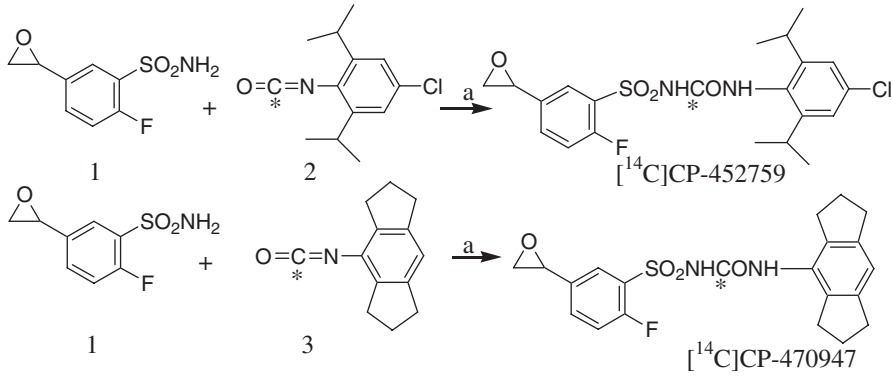
Fig. 1. Glyburide and Diarylsulfonylurea (DASU) IL-1 inhibitor IC_{50}S

cytokine is believed to play a major role in inflammatory disease; thus, modulation of IL-1 is an attractive target for drug research. In an *in vitro* assay,[4] where maturation and release of IL-1 is induced by ATP-treatment of human monocytes, we[5] and others[6] have shown that glyburide is inhibitory. Extensive SAR studies around glyburide led to the discovery of potent DASU inhibitors, exemplified by CP-424,174,[5] that blocked processing and release of IL-1. However, the mechanism by which these agents blocked stimulus-coupled processing was not known. A radiolabeled covalent DASU inhibitor (a radiolabeled inhibitor containing a reactive functionality) was therefore critical for pharmacologic studies aimed at identifying DASU binding proteins involved in the inhibition of IL-1 post-translational processing. This paper describes the synthesis of two [^{14}C]-labeled DASU epoxide inhibitors, CP–452,759 and CP-470,947, which should facilitate identification and characterization of DASU binding proteins involved in the inhibition of processing and release of IL-1 from human monocytes (Figure 1).

Results and discussion

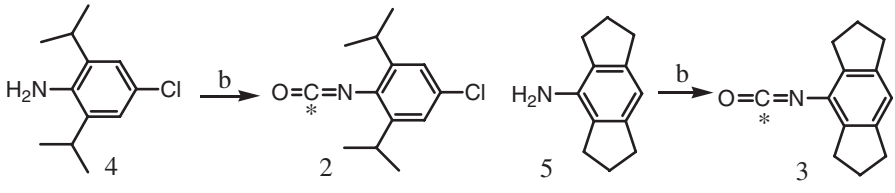
Preparation of the [^{14}C]DASUs (Scheme 1)

[^{14}C]CP-452,759 (0.84 mCi at 55 mCi/mmol) and [^{14}C]CP-470,947 (0.58 mCi, 55 mCi/mmol) were prepared by treatment of excess



(a) NaH/THF
* position of label

Scheme 1.



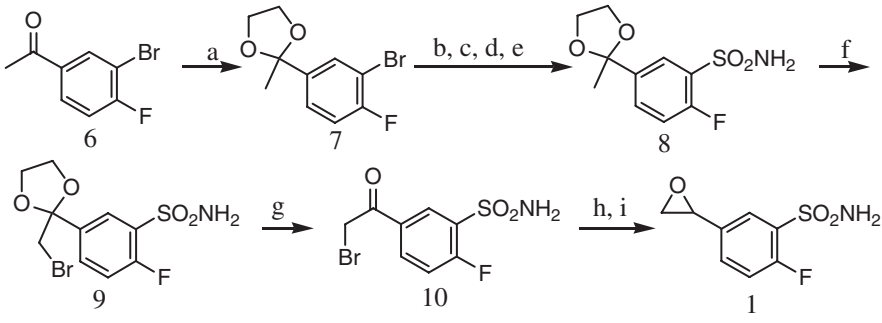
(b) [^{14}C]phosgene/TEA/toluene
*position of label

Scheme 2.

2-fluoro-5-oxiranyl-benzenesulfonamide (**1**) with 5-chloro-2-[^{14}C]-isocyanato-1,3-diisopropylbenzene (**2**) (1.1 mCi) and 4-[^{14}C]-isocyanato-1,2,3,5,6,7-hexahydro-s-indacene (**3**) (0.9 mCi), respectively, using sodium hydride in THF.

Preparation of [^{14}C]isocyanates (Scheme 2)

Treatment of commercially available 2,6-diisopropylphenylamine with NCS/DMF gave **4** in quantitative yield. Treatment of [^{14}C]phosgene (5 mCi at 55 mCi/mmol) with triethylamine and excess 4-chloro-2,6-diisopropylphenylamine (**4**) in warm toluene for 1 hr gave 5-chloro-2-[^{14}C]-isocyanato-1,3-diisopropylbenzene (**2**) in quantitative yield (5 mCi). Longer reaction times lead to lower yields.



(a) TsOH, ethylene glycol/toluene, (b) BuLi/THF, (c) SO_2, (d) NCS/CH_2Cl_2, (e) NH_4OH (f) phenyltrimethylammonium tribromide/CH_3CN, (g) aqueous HCl/dioxane, (h) $NaBH_4$/MeOH, (i) NaOH

Scheme 3.

1,2,3,5,6,7-Hexahydro-sindacen-4-ylamine (**5**)[7] was more reactive, and when treated with [^{14}C]phosgene (35 mCi at 55 mCi/mmol) in toluene, the reaction was complete in 45 min at room temperature. Evaporation of the solvent *in vacuo* resulted in the isolation of significant amounts of a white solid, which did not dissolve in hexanes. After silica gel filtration, 9 mCi of [^{14}C] **3** was isolated (>99% radiochemically pure by radio-TLC).

The synthesis of 2-fluoro-5-oxiranyl-benzenesulfonamide (**1**) is shown in Scheme 3. Dean-Stark distillation of a toluene solution of commercially available 1-(3-bromo-4-fluoro-phenyl)-ethanone (**6**), ethylene glycol and a trace of *p*-toluenesulfonic acid gave 2-(3-bromo-4-fluoro-phenyl)-2-methyl-[1,3]dioxolane (**7**). Successive treatment of **7** with *n*-butyllithium in tetrahydrofuran at −78°C, sulfur dioxide, *N*-chlorosuccinimide in dichloromethane and aqueous ammonium hydroxide gave 2-fluoro-5-(2-methyl-[1,3]dioxolan-2-yl)-benzenesulfonamide (**8**). Bromination of **8** with phenyltrimethyl-ammonium tribromide in acetonitrile gave 5-(2-bromomethyl-[1,3]dioxolan-2-yl)-2-fluoro-benzenesulfonamide (**9**) which was treated with aqueous hydrochloric acid in dioxane to give 5-bromoacetyl-2-fluoro-benzenesulfonamide (**10**). Reduction of **10** with sodium borohydride in methanol, followed by treatment with dilute sodium hydroxide gave **1**.

Experimental

Melting points were taken on a Thomas–Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on

Bruker (AM 300) or Varian (XL250 or T69) instruments. Routine mass spectral data were obtained using either a Hewlett-Packard 5989 MS Engine operated with a particle beam interface and ammonia chemical ionization (PB/CI) or a VG/Fisons Instruments Platform II spectrometer operating with an atmospheric pressure chemical ionization (APCI) source. [^{14}C] Phosgene was purchased from Amersham. All reactions were run under a nitrogen atmosphere.

*2-(3-Bromo-4-fluoro-phenyl)-2-methyl-[1,3]dioxolane (**7**)*

A solution of 1-(3-bromo-4-fluoro-phenyl)-ethanone (**6**) (25 g, 0.115 mol) (Fluorochem), ethylene glycol (20 ml, 0.345 mol) and a trace of *p*-toluenesulfonic acid in 200 ml of toluene was heated at reflux for 12 h. with Dean–Stark separation of water. The reaction was cooled to room temperature, washed with a dilute sodium bicarbonate solution, dried over anhydrous Na_2SO_4 and the solvent was evaporated to give 30 g of **7** (100%) as an oil • TLC: CH_2Cl_2 single spot material R*f* 0.40 • ^{1}H-NMR (300 MHz, DMSO-D_6) δ: (1.52, s, 3 H), (3.67, m, 2 H), (3.95, m, 2 H), (7.34, m, 1 H), (7.40, m, 1 H), (7.63, m, 1 H).

*2-Fluoro-5(2-methyl-[1,3]dioxolan-2-yl)-benzenesulfonamide (**8**)*

n-BuLi [1.6 M in hexane, 50 ml (0.08 mol)] was added dropwise to a solution of 2-(3-bromo-4-fluoro-phenyl)-2-methyl-(1,3)dioxolane (**7**) (20.88 g, 0.08 mol) in 200 ml THF at −78°C. After stirring for 2 h at −78°C, SO_2 was bubbled in for 15 min. The reaction was allowed to warm to room temperature and stirred overnight. A solution of NCS (10.8 g, 0.08 mol) in 150 ml of CH_2Cl_2 was added and the reaction stirred for 1 h. The volatiles were evaporated *in vacuo*, and the brown residue was slurried with CH_2Cl_2 and filtered. The filtrate was treated with 200 ml of 30% NH_4OH and stirred for 3 h. The CH_2Cl_2 layer was separated, dried over anhydrous Na_2SO_4 and evaporated. The residue was triturated with CH_2Cl_2 and filtered to give 8.1 g of **8** (39%); mp 149–150°C • TLC: CH_2Cl_2/MeOH:9/1 v/v single-spot material R*f* 0.5 • ^{1}H NMR (300 MHz, DMSO-D_6) δ: (1.60, s, 3 H), (3.70, m, 2 H), (4.05, m, 2 H), (7.41, t, 1 H), (7.71, broad s, 3 H), (7.84, dd, 1 H).

*5-(2-Bromomethyl-[1,3]dioxolan-2-yl)-2-fluoro-benzenesulfonamide (**9**)*

A solution of phenyltrimethylammonium tribromide (8.4 g, 0.0225 mol) in 20 ml of CH_3CN was added dropwise to a solution of 2-fluoro-5

(2-methyl-[1,3]dioxolan-2-yl)-benzenesulfonamide (**8**) (5.2 g, 0.022 mol) in 100 ml of CH_3CN. The reaction was stirred at room temperature for 1 h. and the solvent was evaporated *in vacuo*. The residue was dissolved in EtOAc, washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/Et_2O:9/1 v/v to give 4.6 g of **9** (62%) as an oil • TLC: CH_2Cl_2/Et_2O:9/1 v/v single-spot material R*f* 0.30 • ^{1}H NMR (300 MHz, $CDCl_3$) δ: (3.68, s, 2 H), (3.80, m, 2 H), (4.21, m, 2 H), (5.15, broad s, 2 H), (7.26, t, 1 H), (7.75, m, 1 H), (8.15, dd, 1 H).

*5-Bromoacetyl-2-fluoro-benzenesulfonamide (**10**)*

A solution of 5-(2-bromomethyl-[1,3]dioxolan-2-yl)-2-fluoro-benzenesulfonamide (**9**) (1.5 g, 4.4 mmol) in 50 ml of dioxane and 10 ml of 2N HCl was heated at reflux for 5 h then cooled to room temperature. The solvent was evaporated *in vacuo* and the residue dissolved in EtOAc, washed with H_2O and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography on silica gel eluting with EtOAc to give 900 mg of material which was recrystallized from MeOAc to give 700 mg **10** (54%); mp 153–158°C • TLC: CH_2Cl_2/Et_2O:7/3 v/v single-spot material R*f* 0.60 • ^{1}H NMR (300 MHz, DMSO-D_6) δ: (5.20, s, 2 H), (7.63, t, 1 H), (7.86, broad s, 2 H), (8.32, m, 2 H).

*2-Fluoro-5-oxiranyl-benzenesulfonamide (**1**)*

A solution of 5-bromoacetyl-2-fluoro-benzenesulfonamide (**10**) (0.5 g, 1.7 mmol) in 20 ml MeOH was cooled to 0°C. $NaBH_4$ (26 mg, 0.67 mmol) was added and the reaction was stirred at 0°C for 15 min. Four milliliter of 1N NaOH was added and the reaction was stirred at 0°C for 4 h. The reaction was adjusted to pH 6 by addition of dilute HCl. The volatiles were evaporated and the residue dissolved in EtOAc, washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated. The product was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH:9/1 v/v to give 180 mg of **1** (48%); mp 100–103°C • TLC: CH_2Cl_2/MeOH:9/1 v/v single-spot material R*f* 0.65 • ^{1}H-NMR (300 MHz, DMSO-D_6) δ: (2.82, m, 1 H), (3.14, m, 1 H), (4.06, m, 1 H), (7.40, t, 1 H), (7.56, m, 1 H), (7.66, m, 3 H).

*4-Chloro-2-isocyanato-1,3-diisopropyl-benzene (**2**) (non-labeled)*

To a solution of 4-chloro-2,6-diiopropyl-phenylamine (**4**) (8.9 g, 0.042 mol) and TEA (4.66 g, 0.046 mol) in 125 ml of THF was added a

1.93 M solution of phosgene in toluene (23.8 ml, 0.046 mol). The reaction was stirred at room temperature for 15 min, then at 70°C for 15 min. The solvents were evaporated *in vacuo*. The residue was dissolved in hexane and purified on a plug of silica gel eluting with 10% v/v CH_2Cl_2/hexane to give 9.45 g of non-labeled **2** (95%) as an oil • TLC: Hex/CH_2Cl_2:2/1 v/v single spot material R*f* 0.70 • ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (1.20, d, 12 H), (3.19, septet, 2 H), (7.06, s, 2 H).

*4-Isocyanato-1,2,3,5,6,7-hexahydro-s-indacene (**3**) (non-labeled)*

To a solution of 1,2,3,5,6,7-hexahydro-s-indacen-4-ylamine (**5**)[7] (1.77 g, 0.01 mol) and TEA (1.11 g, 0.011 mol) in 25 ml of THF was added a 1.93 M solution of phosgene in toluene (5.7 m, 0.011 mol). The reaction was stirred at room temperature for 15 min and at 70°C for 15 min. The solvents were evaporated *in vacuo*. The residue was dissolved in hexane and purified on a plug of silica gel eluting with 10% v/v CH_2Cl_2/hexane to give 1.79 g of non-labeled **3** (90%); • mp 40–41°C • TLC: Hex/CH_2Cl_2:2/1 v/v single-spot material R*f* 0.70 • ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (2.13, t, 4 H), (2.85, m, 8 H), (6.93, s, 1 H).

CP-452,759 (non-labeled)

Sodium hydride [60% dispersion in mineral oil, (23 mg, 0.57 mmol)] was added to a solution of 2-fluoro-5-oxiranyl-benzenesulfonamide (**1**) (113 mg, 0.52 mmol) and 4-chloro-2-isocyanato-1,3-diisopropyl-benzene (**2**) (136 mg, 0.57 mmol) in 10 ml of THF. After stirring at room temperature for 3 h, the solvent was evaporated *in vacuo*. The residue was acidified with 2N HCl and extracted with EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH:9/1 v/v to give 190 mg of CP-452,759 (81%); mp 126–130°C • TLC: CH_2Cl_2/MeOH:9/1 v/v single-spot material R*f* 0.70 • ^{1}H-NMR (300 MHz, DMSO-D_6) δ: (0.97, m, 12 H), (2.76, m, 1 H), (2.93, m, 1 H), (3.10, m, 1 H), (4.05, m, 1 H), (7.04, s, 2 H), (7.35, m, 1 H), (7.50, m, 1 H), (7.77, m, 2 H).

CP-470,947 (non-labeled)

Sodium hydride [60% dispersion in mineral oil, 30 mg (0.75 mmol)] was added to a solution of 2-fluoro-5-oxiranyl-benzenesulfonamide (**1**)

(148 mg, 0.68 mmol) and 4-isocyanato-1,2,3,5,6,7-hexahydro-s-indacene (**3**) (150 mg, 0.75 mmol). After stirring at room for 12 h, the solvent was evaporated *in vacuo*. The residue was acidified with 2N HCl, then extracted with EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was triturated with hexane and filtered to give 210 mg of CP-470,947 (75%); mp 97–102°C • TLC: CH_2Cl_2/MeOH:9/1 v/v single-spot material R*f* 0.65 • ^{1}H-NMR (300 MHz, DMSO-D_6) δ : (1.96, m, 4 H), (2.47, m, 4 H), (2.74, m, 5 H), (3.11, m, 1 H), (4.06, m, 1 H), (6.90, s, 1 H), (7.49, t, 1 H), (7.61, m, 1 H), (7.78, dd, 1 H), (8.05, s, 1 H).

*4-Chloro-2-[^{14}C]-isocyanato-1,3-diisopropylbenzene (**2**)*

4-Chloro-2,6-diisopropylphenylamine (**4**) hydrochloride (0.045 g, 0.18 mmol, 2 eq) was dispersed in ether, and triethylamine (57 µl, 0.41 mmol, 4.5 eq) was added. The solution was filtered through a cotton plug, and most of the ether evaporated *in vacuo*. The residual clear colorless oil was taken up in dry toluene (3 ml) and fitted to a vacuum manifold. The solution was degassed, cooled in a liquid nitrogen bath and evacuated. An ampoule containing [^{14}C]phosgene gas (5 mCi at 55 mCi/mmol) was fitted to the manifold, and the system evacuated. The manifold was then isolated from the pump, the ampoule broken, and the [^{14}C]phosgene allowed to vacuum transfer into the cooled reaction vessel. After 20 min, the cold bath was removed, and the reaction mixture warmed to room temperature. It was then backflushed with nitrogen, and heated to 80°C for 1 h, after which time, no starting material remained (radio-TLC). The mixture was cooled to room temperature and concentrated *in vacuo*. The yellow oil was dissolved in hexanes and filtered through a small plug of silica gel (hexanes) to give 5 mCi 5-chloro-2-[^{14}C]-isocyanato-1,3-diisopropylbenzene (**2**), >99% radiochemically pure by radio-TLC (hexanes).

*4-[^{14}C]-isocyanato-1,2,3,5,6,7-hexahydro-s-indacene (**3**)*

[^{14}C]Phosgene (35 mCi at 57 mCi/mmol) as a solution in toluene, 35 ml was cooled to 0°C, and triethylamine (0.50 ml, 3.5 mmol, 6 eq) and 1,2,3,5,6,7-hexahydro-s-indacen-4-ylamine (**5**)[7] (0.17 g, 0.1 mmol, 1.6 eq) were added as a solution in toluene (2 ml). After 15 min, the reaction was removed from the ice bath and let warm to room temperature over 30 min after which time radio-TLC analysis indicated desired product

was present in 93%. The reaction mixture was concentrated *in vacuo*, dissolved in hexanes and filtered through a plug of silica gel (hexanes). Concentration of the filtrate gave 9 mCi 4-[^{14}C]-isocyanato-1,2,3,5,6,7-hexahydro-s-indacene (**3**), which was >99% pure by radio-TLC (hexanes).

[^{14}C]-CP-452,759

The 5-Chloro-2-[^{14}C]-isocyanato-1,3-diisopropylbenzene (**2**) (1.08 mCi at 55 mCi/mmol) and 2-fluoro-5-oxiranylbenzenesulfonamide (**1**) (95 mg, 0.022 mmol, 1.1 eq) were dissolved in freshly distilled THF and sodium hydride (2 mg) was added. After 5 min, radio-TLC indicated complete reaction. The reaction mixture was concentrated *in vacuo*, dissolved in water (5 ml) and extracted with ether. The aqueous phase was then acidified with 2N HCl, and extracted with ethyl acetate. The combined ethyl acetate layers were dried, filtered, concentrated and the residue chromatographed (silica gel, EtOAc) to give 0.84 mCi [^{14}C]CP-452,759 (>99% pure radio-TLC, EtOAc).

[^{14}C]-CP-470,947

4-[^{14}C]-Isocyanato-1,2,3,5,6,7-hexahydro-s-indacene (**3**) (0.9 mCi at 57 mCi/mmol) was dissolved in THF (5 ml), and 2-fluoro-5-oxiranylbenzenesulfonamide (**1**) (4.2 mg, 0.19 mmol, 1.2 eq) was added, followed by sodium hydride (2 mg). After 15 min, no starting material remained by radio-TLC. The reaction mixture was concentrated *in vacuo*, and the residue was dispersed in 0.2 M HCl (5 ml) and extracted with ethyl acetate (3 × 5 ml). The combined organic layers were dried, filtered, concentrated and the residue chromatographed (EtOAc) to give 0.58 mCi [^{14}C]CP-470,947 (>99% pure by radio-TLC, EtOAc).

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