Chemical Research in Toxicology

DECEMBER 2001
VOLUME 14, NUMBER 12

Articles

Synthesis of New Carboxylesterase Inhibitors and Evaluation of Potency and Water Solubility

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Received April 6, 2001

Carboxylesterases are essential enzymes in the hydrolysis and detoxification of numerous pharmaceuticals and pesticides. They are vital in mediating organophosphate toxicity and in activating many prodrugs such as the chemotherapeutic agent CPT-11. It is therefore important to study the catalytic mechanism responsible for carboxylesterase-induced hydrolysis, which can be accomplished through the use of potent and selective inhibitors. Trifluoromethyl ketone (TFK)-containing compounds are the most potent esterase inhibitors described to date. The inclusion of a thioether moiety β to the carbonyl further increased TFK inhibitor potency. In this study, we have synthesized the sulfone analogues of a series of aliphatic and aromatic substituted thioether TFKs to evaluate their potency and solubility properties. This structural change shifted the keto/hydrate equilibrium from <9% hydrate to >95% hydrate, forming almost exclusively the *gem*-diol. These new compounds were evaluated for their inhibition of carboxylesterase activity in three different systems, rat liver microsomes, commercial porcine esterase, and juvenile hormone esterase in cabbage looper (*Trichoplusia ni*) hemolymph. The most potent inhibitor of rat liver carboxylesterase activity was 1,1,1-trifluoro-3-(decane-1-sulfonyl)-propan-2,2-diol, which inhibited 50% of the enzyme activity (IC_{50}) at 6.3 ± 1.3 nM and was 18-fold more potent than its thioether analogue. However, the sulfone derivatives were consistently poorer inhibitors of porcine carboxylesterase activity and juvenile hormone esterase activity, with IC_{50} values ranging from low micromolar to millimolar. The compound 1,1,1-trifluoro-3-(octane-1-sulfonyl)-propan-2,2-diol was shown to have a 10-fold greater water solubility than its thioether analogue, 1,1,1-trifluoro-3-octylsulfanyl-propan-2-one (OTFP). These novel compounds provide further evidence of the differences between esterase orthologs, suggesting that additional development of esterase inhibitors may ultimately provide a battery of ortholog and/or isoform selective inhibitors analogous to those available for other complex enzyme families with overlapping substrate specificity.

Introduction

Esterases are α/β-fold hydrolase enzymes that catalyze the hydrolysis of both endogenous and exogenous esters. Each member of this group of enzymes contains a similar core comprised of an α/β-sheet of eight β-sheets connected by α-helices. The α/β-hydrolase family was first described by Ollis et al. and covers a broad range of enzymes that share similarities in secondary and tertiary structures (*1*). These enzymes are related by divergent evolution and

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10.1021/tx015508+ CCC: $20.00
Published on Web 10/27/2001

include lipases, esterases, cholinesterases, haloalkane dehalogenases, and epoxide hydrolases (*2*). The sequence identities of esterases of the α/*β*-hydrolase family vary widely, but their mechanisms are all centered around a catalytic triad of which the most common set is Ser-His-Glu(Asp) (*3*). Recent work has shown the potential for a second serine as a fourth catalytic residue (*4*). Together, this group of enzymes plays an essential role in the activation, metabolism, and detoxification of numerous compounds of biological importance.

Carboxylesterases (EC 3.1.1.1) are a subset of the α/*β*-hydrolase family and have very broad substrate selectivity. They are B-esterases under the nomenclature of Aldridge by virtue of their inhibition by organophosphates (*5*) and are characterized by their catalysis of the hydrolysis of a carboxylester to the corresponding alcohol and acid (*6*). Carboxylesterases are important in the metabolism of many exogenous compounds including pesticides and pharmaceuticals, but their endogenous role is not well understood (*7*). Carboxylesterase-mediated hydrolysis is used in the design of many drugs and pharmacophores. For example, the hydrolysis of ester-containing prodrugs has been employed in the development of the chemotherapeutic agents CPT-11 (*8*, *9*) and 10-hydroxycamptothecin fatty acid esters (*10*), and in the activation of the blood cholesterol drug lovastatin (*11*). In addition, carboxylesterases are of importance in the transesterification of meperidine (Demerol) and methylphenidate (Ritalin) (*12*), and are essential in the hydrolysis of cocaine and heroin (*13*, *14*), as well as aspirin (*15*, *16*).

Carboxylesterases are also important in the hydrolysis and subsequent detoxification of pyrethroid (*17*), carbamate (*18*) and organophosphate (*19*) insecticides. Carboxylesterases detoxify carbamates and organophosphates by hydrolyzing ester-containing bonds and by acting as an inhibitor sink, thus preventing these compounds from inhibiting acetylcholinesterase (*20*). In mammals, the carboxylesterase-catalyzed hydrolysis of pyrethroids is one of the main routes of detoxification of these compounds, along with oxidase activity, and is responsible for their relatively low mammalian toxicity (*21*). In addition, inhibition of carboxylesterases has been shown to synergize both pyrethroid and organophosphate toxicity (*20*, *22*). One of the major strategies of pesticide development exploits the fact that mammals contain higher levels of carboxylesterases compared to insects and are thus able to more quickly detoxify and excrete the compounds (*21*). Carboxylesterases are also of importance in pest resistance management, with pyrethroid (*23*), carbamate (*24*) and organophosphate (*25*, *26*) resistant strains of many insects showing elevated levels of carboxylesterase.

Juvenile hormone esterases (JHEs)[1] are insect carboxylesterases that are highly substrate selective (*27*). Unlike other carboxylesterases, their endogenous function is well understood, and they appear to have little to no exogenous function (*28*). JHEs hydrolyze insect juvenile hormone (JH), which in turn regulates molting in some insects (*29*). JHE is a key enzyme in the development of many common crop pests and is a target of biological pesticides (*30*). Molting states of insects can be disrupted through JHE inhibition, resulting in alteration of larval feeding habits (*31*). The selectivity of JHE for the methyl ester of JH is rare among esterases (*29*). In particular, the JHEs studied are ineffective at hydrolyzing *p*-nitrophenyl acetate (PNPA), one of the most ubiquitous esterase substrates used for experimental determination of esterlytic activity (*27*).

Several recent esterase reviews have been conducted, all of which discuss the inhibition of esterases by trifluoromethyl ketones (TFK), a group of extremely potent transition state analogue inhibitors (*1*, *2*, *7*, *32*). Polarized ketones undergo hydration, achieving equilibrium between their keto and hydrate forms (*30*). This hydration shifts the geometry from trigonal planar to tetrahedral, thus mimicking the transition state of the bound substrate (*33*) and forming a stable analogue (*34*). The binding of an enzyme to the ketone moiety also results in a tetrahedral geometry. However, due to the lack of a cleavable bond, the enzyme is inhibited, resulting in the potency of this group of compounds (*30*). TFKs have been extensively studied for their esterase inhibition properties and have been widely used to probe the catalytic mechanism of numerous members of the α/*β*-fold hydrolase family (*2*, *30*).

Roe et al. (*35*) reported the synthesis of a new TFK derivative of increased potency and water solubility. This new compound reputedly had a 3-fold increase in JHE inhibition potency over 1,1,1-trifluoro-3-octylsulfanyl-propan-2-one (OTFP, **2a**) in the lepidopteran cabbage looper (*Trichoplusia ni*) (*35*). Roe et al. achieved this increase in inhibition potency by oxidizing the sulfur atom *β* to the carbonyl of the TFK to the corresponding sulfone. This chemical change also served to drive the *gem*-diol/ketone equilibrium to favor the *gem*-diol. The combination of these two effects should drastically increase the water solubility of the compound. It therefore appeared to be an anomaly, a compound of increased potency and increased water solubility. The most common potent inhibitors of JHE are long-chain aliphatic TFK derivatives that are hydrophobic compounds with log *P* values on the order of 3−5 (*30*). Therefore to investigate this phenomenon, we synthesized a series of sulfone analogues from a set of thioether-containing TFKs and evaluated their inhibition potency against juvenile hormone esterase activity in *Trichoplusia ni* hemolymph to compare with Roe et al. (*35*). We then investigated the ability of these new compounds to inhibit esterlytic activity in rat liver microsomal preparations and crude commercial porcine liver esterase. Last, we assessed the water solubility of the new sulfone compounds versus their thioether analogues.

Experimental Procedures

CAUTION! *The hydroxyiodinane oxide precursor of the Dess−Martin periodinane has been reported to be explosive on impact or in conditions over 200 °C (36, 37). We found that Dess−Martin periodinane purchased from T. C. I. America Inc. was stable but that synthesis of the compound always resulted in at least a trace amount of the precursor oxide, which was indeed explosive.*

[1] Abbreviations: BSA, bovine serum albumin; DMF, dimethylformamide; DMP, Dess−Martin periodinane; ESI-MS, electrospray ionization MS; FID, flame ionization detector; IC_{50}, 50% inhibition concentration; JH, juvenile hormone; JHE, juvenile hormone esterase; *m*-CBA, *m*-chlorobenzoic acid; *m*-CPBA, *m*-chloroperoxybenzoic acid; OTFP, 1,1,1-trifluoro-3-octylsulfanyl-propan-2-one; OTFPdOHSO$_2$, 1,1,1-trifluoro-3-(octane-1-sulfonyl)-propan-2,2-diol; OTFPmOH, 1,1,1-trifluoro-3-octylsulfanyl-propan-2-ol; OTFPmOHSO$_2$, 1,1,1-trifluoro-3-(octane-1-sulfonyl)-propan-2-ol; PNPA, *p*-nitrophenyl acetate; PTU, phenylthiourea; QSAR, quantitative structure−activity relationship; *sat*, saturated; TFK, trifluoromethyl ketone; *Trichoplusia ni*, *T. ni*.

Table 1. Physical and Structural Data for All Synthesized Compounds

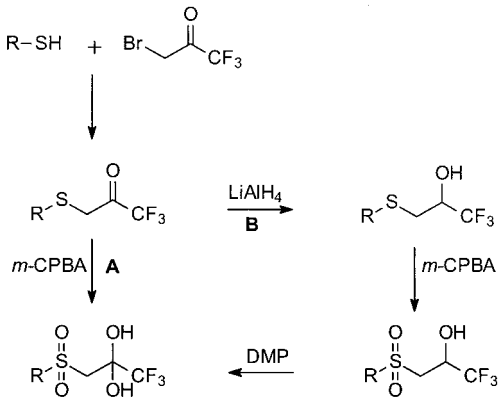
compd	melting point (°C)	GC-MS *m/z* (relative intensity)	^{1}H NMR	^{19}F NMR	IR (neat, cm^{-1})
1b ($C_9H_{17}F_3O_4S$)	85−87	261 (39%, $[M-OH]^+$); 177 (3%, $[M-C_6H_{13}O]^+$); 85 (63%, $[M-C_3H_4F_3O_4S]^+$); 69 (76%, $M-C_4H_8F_3O_4S]^+$); 55 (100%, $[M-C_5H_{10}F_3O_4S]^+$)	δ 0.90 (t, 3H, J = 6.45 Hz), 1.32 (br m, 4H), 1.46 (m, 2H), 1.86 (m, 2H), 3.33 (m, 2H), 3.39 (s, 2H), 4.90 (br s, 1.5H, D_2O exchangeable)	−87.31 (s, 97%) −79.21 (s, 3%)	1123.7, 1189.5, 1310.4, 2868.2, 2953.5, 3443.3, 3489.1
2b ($C_{11}H_{21}F_3O_4S$)	87−88	289 (11%, $[M-OH]^+$); 177 (6%, $[M-C_8H_{17}O]^+$); 113 (2%, $[M-C_3H_2F_3O_3S]^+$); 69 (100%, $[M-C_6H_{12}F_3O_4S]^+$); 55 (58%, $[M-C_7H_{14}F_3O_4S]^+$)	δ 0.88 (t, 3H, J = 6.45 Hz), 1.29 (br m, 8H), 1.45 (m, 2H), 1.87 (m, 2H), 3.33 (m, 2H), 3.39 (s, 2H), 4.93 (br s, 1.5H, D_2O exchangeable)	−87.29 (s, 97%) −79.21 (s, 3%)	1133.2, 1179.4, 1303.9, 2861.0, 2927.7, 3439.4, 3523.9
3b ($C_{13}H_{25}F_3O_4S$)	waxy solid	317 (10%, $[M-OH]^+$); 177 (2%, $[M-C_{10}H_{21}O]^+$); 205 (5%, $[M-C_3H_4F_3O_2]^+$); 111 (17%, $[M-C_5H_{10}F_3O_4S]^+$); 97 (44%, $[M-C_6H_{12}F_3O_4S]^+$); 83 (97%, $[M-C_7H_{14}F_3O_4S]^+$); 69 (82%, $[M-C_8H_{16}F_3O_4S]^+$); 55 (100%, $[M-C_9H_{18}F_3O_4S]^+$)	δ 0.88 (t, 3H, J = 6.45 Hz), 1.27 (br m, 12H), 1.46 (m, 2H), 1.87 (m, 2H), 3.34 (m, 2H), 3.39 (s, 2H), 4.88 (br s, 1.5H, D_2O exchangeable)	−87.31 (s, 96%) −79.21 (s, 4%)	1124.3, 1189.0, 1309.9, 2860.0, 2926.7, 3435.9, 3491.8
4b ($C_{15}H_{29}F_3O_4S$)	waxy solid	345 (21%, $[M-OH]^+$); 177 (2%, $[M-C_{12}H_{25}O]^+$); 233 (4%, $[M-C_3H_4F_3O_2]^+$); 125 (12%, $[M-C_6H_{12}F_3O_4S]^+$); 111 (60%, $[M-C_7H_{14}F_3O_4S]^+$); 97 (96%, $[M-C_8H_{16}F_3O_4S]^+$); 83 (84%, $[M-C_9H_{18}F_3O_4S]^+$); 69 (100%, $[M-C_{10}H_{20}F_3O_4S]^+$); 55 (42%, $[M-C_{11}H_{22}F_3O_4S]^+$)	δ 0.88 (t, 3H, J = 6.45 Hz), 1.26 (br m, 16H), 1.43 (m, 2H), 1.87 (m, 2H), 3.33 (m, 2H), 3.39 (s, 2H), 4.88 (br s, 1.5H, D_2O exchangeable)	−87.31 (s, 95%) −79.21 (s, 5%)	1133.2, 1179.4, 1310.4, 2854.4, 2924.6, 3461.0, 3497.9
5b ($C_{11}H_{13}F_3O_4S$)	96−98	105 (100%, $[M-C_3H_5F_3O_4S]^+$); 91 (5%, $[M-C_4H_7F_3O_4S]^+$); 77 (16%, $[M-C_5H_9F_3O_4S]^+$)	δ 3.18 (m, 2H), 3.30 (s, 2H), 3.64 (m, 2H), 4.85 (br s, 1.5H, D_2O exchangeable), 7.32 (m, 5H)	−87.29 (s, 95%) −79.21 (s, 5%)	1121.4, 1188.7, 1430.1, 3503.9

Chemicals. Reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) unless otherwise noted and were used without further purification. Initial purity of all synthesized compounds was assessed by 10 cm F_{254} silica TLC plates (EM Science; Gibbstown, NJ) using either a chloroform/ethanol (95:5) or hexane/ethyl acetate (70:30) mixture and visualized with phosphomolybdic acid and heat. Purity was assayed, in part, by the number of spots responding to phosphomolybdic acid and by a lack of UV sensitive spots at 254 nm, confirming removal of UV-active impurities. To further gauge purity, dilutions were analyzed on a Hewlett-Packard 5890A GC (Palo Alto, CA) equipped with a flame ionization detector (FID) and a 15 m × 0.25 mm i.d., 0.25 μm DB-5 column (J&W Scientific; Folsom, CA). The temperature program was initiated at 100 °C for a 1 min hold and then ramped at 20 °C/min to 300 °C and held for 5 min. All compounds were of a purity of >97% by GC and NMR and were one spot on TLC. Due to the hygroscopic nature of these compounds, it was not possible to acquire accurate elemental analyses. This problem was also reported by Nair et al. (*38*). Melting point were determined with a Thomas-Hoover apparatus (A. H. Thomas Co., Philadelphia, PA) and are uncorrected.

Structural support was provided by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR (Varian 300 MHz; Palo Alto, CA), IR (Mattson Galaxy Series FTIR 3000, Madison, WI), and a Saturn 4D Ion Trap mass spectrometer coupled to a Cx3400 GC (Varian; Sugarland, Texas) and is listed in Table 1. Electrospray mass spectral data was collected on a Quatro BQ (Fisons; Altracham, England).

Chemical Synthesis Of Sulfone Derivatives, Method A. Two different synthetic routes were used to synthesize the sulfone derivatives and are outlined in Scheme 1. Method A is a new synthetic procedure that reduces the synthesis scheme of Roe et al. (*35*) to two steps. The synthesis of 1,1,1-trifluoro-3-(octane-1-sulfonyl)-propan-2,2-diol (OTFPdOHSO$_2$, **2b**) is given as an example. First, 1,1,1-trifluoro-3-octylsulfanyl-propan-2-one (OTFP, **2a**) was synthesized with methods of Ashour et al. (*39*). Briefly, 3-bromo-1,1,1-trifluoroacetone (75 mmol, 7.8 mL) was added to a stirring solution of octylthiol (75 mmol, 13 mL) in CCl_4 (10 mL). The reaction was allowed to proceed under a

Scheme 1. Synthesis Scheme for Sulfone Derivatives of Trifluoromethyl Ketones (TFK)[a]



R	thioether	sulfone
hexyl	**1a**	**1b**
octyl	**2a**	**2b**
decyl	**3a**	**3b**
dodecyl	**4a**	**4b**
phenethyl	**5a**	**5b**

[a] Method A is a novel procedure developed for this paper. Method B is based upon the work of Roe et al. (*35*). Compounds are numbered in two sets, all thioether TFKs are referred to as "thioether", and all sulfone TFK derivatives are "sulfone". DMP refers to the Dess−Martin periodinane (*36*).

gentle stream of N_2 and was monitored by GC/FID. The reaction was considered to be complete at 24 h (75% completion) and 25 mL of diethyl ether was added. The entire mixture was then washed with 2 × 25 mL of aqueous sodium bicarbonate (saturated, *sat*), and the aqueous phase was extracted with 2 × 25 mL of diethyl ether. The organic phases were collected, dried over magnesium sulfate, and filtered, and the solvent was stripped by rotary vacuum evaporation. The crude mixture was vacuum distilled (bp 85 °C, 1.5 Torr) to yield the purified product (>97% purity by GC): ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.45 Hz), 1.26 (br m, 10H), 1.57 (m, 2H), 2.50 (t, 2H, J = 7.04 Hz), 3.48 (s, 2H); ^{19}F NMR ($CDCl_3$) δ −76.22 (s, 91%), −85.83 (s, 90%); IR (neat) cm^{-1} 1156.5, 1204.5, 1750.3, 2861.0, 2929.1; single spot by TLC (hexanes:ethyl acetate, 70:30).

Compound **2b** was formed by adding **2a** (1.12 mmol, 0.286 g) to a stirring solution of *m*-chloroperoxybenzoic acid (*m*-CPBA, 70−75%, 1.4 g) in dichloromethane. After stirring for 48 h, the reaction was worked up using methods adapted from Camps et al. (*40*). Sodium fluoride (23.8 mmol, 1.0 g), which had been activated by heating at 100 °C for 24 h under vacuum (85 Torr), was added to the reaction mixture followed by Celite to create a slurry, which was then filtered through a Celite cake to remove the *m*-chlorobenzoic acid (*m*-CBA). It was also possible to use extensive sodium bicarbonate (*sat*) washes to remove the *m*-CBA. The mixture was then washed with 2 × 50 mL of aqueous sodium bicarbonate (*sat*) and the aqueous phases were extracted with 2 × 50 mL of diethyl ether. The reaction wash was monitored by GC/FID for the presence of *m*-CBA following the reaction and additional sodium bicarbonate (*sat*) washes were used as necessary to extract any residual acid. Once all acid had been removed, the reaction mixture was dried with magnesium sulfate, filtered, and stripped. The pure product was recrystallized from 1-chlorobutane to give white needlelike crystals (1.05 mmol, 0.320 g, 94% yield): mp 87−88 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.45 Hz), 1.29 (br m, 10H), 1.87 (m, 2H), 3.33 (m, 2H), 3.39 (s, 2H), 4.79 (br s, 1.5H, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ 13.96, 21.72, 22.53, 28.24, 28.41, 28.88, 31.64, 52.57, 56.27, 91.88 (q, J_{CF} = 33 Hz), 121.78 (q, J_{CF} = 286 Hz); ^{19}F NMR ($CDCl_3$) δ −79.21 (s, 3%), −87.29 (s, 97%); IR (neat) cm^{-1} 1133.2, 1179.4, 1303.9, 2861.0, 2927.7, 3439.4, 3523.9; single spot by TLC (chloroform:ethanol, 95:5); Electrospray ionization MS (ESI-MS) *m*/*z* calculated for $[M - H-H_2O]^-$ ($C_{11}H_{18}F_3O_3S$) = 287.1, observed 287.4.

Chemical Synthesis of Sulfone Derivatives, Method B. The second synthetic method was based upon procedures of Roe et al. (*35*). It was however necessary to significantly alter those procedures as published. The synthesis of compound **2b** is given as a sample synthesis for Method B. Compound **2a** was synthesized as described above. 1,1,1-Trifluoro-3-octylsulfanyl-propan-2-ol (OTFPmOH) was formed from methods adapted from Linderman and Graves (*41*). Lithium aluminum hydride (1.85 mmol, 3.7 mL) was added to a stirred solution of **2a** (4.0 mmol, 1.02 g) in diethyl ether at 0 °C, followed by stirring for 1 h after which the reaction was quenched with 10 mL of aqueous sulfuric acid (5%). The mixture was washed with 2 × 20 mL of aqueous sodium bicarbonate (*sat*), followed by extraction with 2 × 20 mL of diethyl ether. The organic phase was dried over magnesium sulfate, filtered, and stripped to give 90% yield by GC/FID (3.7 mmol, 0.933 g). ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.45 Hz), 1.27 (br m, 12H), 1.62 (m, 2H), 2.58 (m, 2H), 3.09 (br s, 1H, D_2O exchangeable), 4.03 (m, 1H); single spot by TLC (hexanes:ethyl acetate, 70:30).

1,1,1-Trifluoro-3-(octane-1-sulfonyl)-propan-2-ol (OTFPmOHSO$_2$) was formed by adding OTFPmOH (3.7 mmol, 0.933 g) to a stirred solution of *m*-CPBA (70−75%, 2.6 g) in dichloromethane. After stirring for 48 h, the product was worked up as for the **2b** synthesis in Method A. The pure product was recrystallized from 1-chlorobutane to give white needlelike crystals (2.33 mmol, 0.677 g, 65% yield): mp 80−81 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.45 Hz), 1.27 (br m, 10H), 1.84 (m, 2H), 3.16 (m, 2H), 3.36 (m, 2H), 3.99 (s, 1H), 4.64 (s, 1H);^{13}C NMR ($CDCl_3$) δ 14.00, 21.68, 22.53, 28.29, 28.92, 31.64, 52.20, 55.28, 66.33 (q, J_{CF} = 32 Hz); 123.98 (q, J_{CF} = 282 Hz); ^{19}F NMR ($CDCl_3$) δ −79.92 (d, J_{HF} = 8.4 Hz); single spot by TLC (chloroform:ethanol, 95:5); ESI-MS *m*/*z* $[M - H]^-$ ($C_{11}H_{20}F_3O_3S$) = 289.1, observed 289.2.

Compound **2b** was formed by the addition of OTFPmOHSO$_2$ (0.79 mmol, 0.229 g) to a stirring solution of the Dess−Martin periodinane (0.16 M; T. C. I. America, Portland, OR) in dichloromethane, which was further stirred for 1 h followed by the addition of 20 mL of diethyl ether. The mixture was then extracted with 60 mL of sodium thiosulfate (0.26 M) in sodium bicarbonate (*sat*). The organic fraction was washed with 2 × 25 mL of sodium bicarbonate (*sat*) and the combined aqueous fractions were extracted with 2 × 50 mL of diethyl ether, dried over magnesium sulfate, filtered, and stripped. The purified product was recrystallized from 1-chlorobutane to give white needlelike crystals (0.28 mmol, 0.084 g, 36% yield). Physical data were identical as for compound **2b** synthesized via Method A.

Juvenile Hormone Esterase Activity. JHE assays were conducted with JHE from *Trichoplusia ni* (*T. ni*). Enzyme preparations were collected from larvae of *T. ni* (Entopath; Easton, PA), which had reached day two of the fifth instar. Larvae were placed on ice to facilitate handling. After 5 min on ice, a single proleg was cut to allow hemolymph to well from the body, which was then harvested as described in Roe et al. (*35*). Briefly, hemolymph was collected into a capillary tube and transferred into microfuge tubes containing ∼0.5 mmol of phenylthiourea (PTU) on ice. The hemolymph was then centrifuged for 5 min at 1000*g* and the supernatant was collected and transferred into a microfuge tube containing 0.1 M phosphate buffer (pH 7.4) and 0.01% PTU for a final 1:1 dilution of hemolymph in buffer. The diluted hemolymph had a specific activity of 1.0 nmol of juvenile hormone III/min/mg of protein and was stored at −80 °C until use.

JHE activity was measured with a partition assay using ^{3}[H]juvenile hormone III (^{3}[H]JH III) (*42*). Substrate was prepared by mixing 80 μL of radiolabeled JH III (10−20 Ci/mmol, 0.1 mCi/mL, >97% pure; New England Nuclear, Boston, MA) with 20 μL of unlabeled JH III (25 mM; Sigma Chemical Co., St Louis, MO) in hexane. The solvents were removed by heating to ∼35 °C under a gentle stream of N_2, then 1 mL of ethanol was added to give a final JH III concentration of 0.5 mM. Hemolymph preparations were diluted 300-fold in sodium phosphate buffer (pH 7.4, 0.1 M, 0.1 mg/mL bovine serum albumin, BSA) and incubated with inhibitors for 10 min at 30 °C. All inhibitor stock solutions were prepared in dimethylformamide (DMF) and the total volume of DMF added to the assay never exceeded 1%, which control studies showed had little effect upon JHE activity. The substrate JH III was then added and vortexed for a final concentration of 5 μM, and the solution was incubated for 15 min at 30 °C. Following incubation, the mixture was extracted with 50 μL of a solution of methanol:water:ammonium hydroxide (10:9:1) and 250 μL of isooctane, followed by vigorous vortexing for 10 s to form an emulsion. Samples were centrifuged for 5 min at 1000*g* and the radioactivity in 50 μL of the aqueous phase was measured by a Wallac 1409 liquid scintillation counter (Wallac; Turku, Finland) according to Hammock and Sparks (*42*).

Mammalian Carboxylesterase Activity. Two sources of mammalian carboxylesterase were used to screen activity, rat liver microsomal preparations, and crude porcine liver carboxylesterase. Assays were conducted in 96-well microtiter styrene flat bottom plates (Dynex Technologies, Inc.; Chantilly, VA) at 30 °C using methods of Huang et al. (*43*). Inhibitor stock solutions were prepared in DMF, and concentrations were chosen such that DMF levels never exceeded 1%. Control studies showed very little effect of DMF upon enzyme activity at these levels. However, all samples were corrected for any potential solvent effects. Enzyme was incubated with the inhibitor for 5 min and then substrate (*p*-nitrophenyl acetate, PNPA) was added for a final concentration of 0.5 mM. After substrate addition, the plate was read kinetically for 2 min with optical density monitored at 405 nm for the appearance of the *p*-

nitrophenolate anion hydrolysis product (Spectramax 200; Molecular Devices, Sunnyvale, CA).

Rat liver microsomal carboxylesterase activity assays were performed with solubilized rat liver microsomes prepared by methods adapted from Huang et al. (*43, 44*). Eight-week-old Fisher 344 rats were sacrificed by carbon dioxide asphyxiation, and livers were excised and perfused with a 1% KCl solution to remove all blood, which contains high levels of serum esterases (*7*). Livers were then pooled, homogenized on ice with a Polytron homogenizer (Brinkmann Instruments; Westbury, NY), and centrifuged at 10000*g* for 20 min at 4 °C. Supernatant fractions were further centrifuged at 100000*g* for 60 min. The resulting pellet was resuspended in a sodium phosphate buffer (pH 7.4, 0.1 M) to give a final protein concentration of 33.5 mg/mL and frozen at −80 °C. Before use in enzyme assays, microsomes were solubilized with 1% *n*-octyl β-D-glucopyranoside (Sigma Chemical Co.) and diluted with sodium phosphate buffer (pH 7.4, 0.1 M) to a final protein concentration of 4.2 mg/mL. Samples were then incubated for1 h, followed by centrifugation at 100000*g* for 1 h, and the supernatant was aliquoted and frozen at −80 °C. For assays, liver microsomal preparations were diluted to a final protein concentration of 0.8 mg/mL in 0.1 M sodium phosphate buffer (pH 7.4) with a specific activity of 67 μmol of *p*-nitrophenyl acetate/min/mg protein. Assays were conducted with 32 mg of protein in a total volume of 200 μL. Protein concentrations were determined using methods of Bradford with BSA as a standard (*45*).

Crude porcine liver carboxylesterase (EC 3.1.1.1) was purchased from Sigma Chemical Co. (catalog no. E-3019). Assays were conducted in a similar format as for rat liver carboxylesterase activity with PNPA as the substrate. Working solutions of enzyme were made-up at 0.02 mg of total protein/mL in sodium phosphate buffer (pH 7.4, 0.1 M) for an activity of 0.3 units/mL (one unit will hydrolyze 1.0 μmol of ethyl butyrate to butyric acid and ethanol at pH 8.0 at 25 °C). Assays were conducted with 0.8 mg of protein in a total volume of 200 μL.

Enzyme Inhibition Determination. The concentration at which 50% of the enzyme activity was inhibited (IC_{50}) was determined identically for all three enzymes. IC_{50}s were calculated from a minimum of five datum points with at least two points above and below the IC_{50} in the linear region of the curve. All assays were run in at least triplicate, with each replicate consisting of an average of 3 individual determinations.

Solubility Determination. Solubility was determined using methods of Nellaiah et al. (*46*), which determine the solubility of the compound based upon its turbidity. Compounds were dissolved in DMF and 10 μL of solution was added to a cuvette containing 1 mL of a sodium phosphate buffer (pH 7.4, 0.1 M) at 25 °C. DMF concentrations therefore never exceeded 1% of the total volume. The absorbance was read at 700 nm on a Cary 100 Bio Spectrophotometer (Varian, Walnut Creek, CA). The concentration of inhibitor that resulted in a significant increase (>4-fold) in absorbance was interpreted as exceeding the solubility of the compound. Solubilities are therefore reported as a range between the concentration that exceeded solubility and the point immediately preceding it.

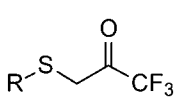
Results and Discussion

Trifluoromethyl ketone-containing compounds are very potent inhibitors of a large number of esterases from a wide variety of sources across different phyla (*7*). It is generally thought that the trifluoromethyl moiety enhances the activity of the inhibitor by further polarizing the ketone, thereby enhancing nucleophilic attack by the nucleophilic hydroxyl group of the serine residue in the active site of the enzyme (*30*). The extreme potency of TFKs is attributed to their ability to act as transition-state analogues. They mimic the structure of the transition-state complex through a theorized tetrahedral geometry in the bound inhibitor (*29*). A number of different TFK-containing compounds have been synthesized, and the structure−activity relationship for this group of compounds has been extensively explored (*30*). It was found that placing a sulfur atom β to the carbonyl greatly increased the potency of the inhibitor. Linus Pauling proposed that sulfur could act as an electronic mimic of unsaturation (*47*). This theory was originally applied to JHE where it was thought that the sulfur would mimic the double bond present in the natural substrate juvenile hormone, thus creating an inhibitor that better mimicked the substrate (*48*). Roe et al. further explored this chemistry by oxidizing the sulfur to a sulfone (*35*). We therefore decided to use this chemistry in the development of new esterase inhibitors to study other carboxylesterases in addition to JHE. The inclusion of a sulfone moiety was intriguing to us, as it appeared to offer both increased potency and water solubility.

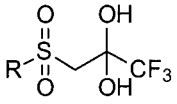
(A) Synthesis. A series of TFK sulfone analogues was synthesized for evaluation as inhibitors of carboxylesterase activity. A single example of this compound class was previously reported by Roe et al. (*35*). We report here the same synthesis in two steps with significantly increased yield and the elimination of a potentially hazardous reagent (Method A, Scheme 1). Due to the inability to efficiently remove the *m*-CBA acid after direct oxidation of the parent thioether TFK with *m*-CPBA, Roe et al. abandoned this reaction for a considerably more difficult reaction scheme (Method B, Scheme 1). However, we found that by using methods of Camps et al. (*40*), we were able to remove all of the *m*-CBA. The cleanup step following the oxidation was the key to successful direct oxidation of the thioether to the sulfone. Camps et al. achieved the removal of *m*-CBA by precipitating the acid with sodium fluoride. This technique followed by extensive washing with sodium bicarbonate (*sat*) served to remove all acid from the system. Sodium carbonate (*sat*) was not any more effective at *m*-CBA removal and attempts to use a stronger base (such as sodium hydroxide) resulted in decomposition of the molecule. By employing the methods of Camps et al., we successfully eliminated all *m*-CBA from the direct oxidation allowing for the isolation of the product by recrystallization in high yield (94%). We then successfully applied this simplified procedure to four additional compounds.

To ensure that the product identity was consistent through this modified procedure, we also repeated the synthesis of $OTFPdOHSO_2$ (**2b**) using methods of Roe et al. (*35*) (Method B in Scheme 1). Overall yields for this route of synthesis were generally low due to the large number of steps required. Roe et al. reported a 57% overall yield from **2a** to **2b**, and we achieved a 22% yield. In addition, we found it necessary to modify several points in the procedure in order to successfully complete the reaction. Again, it was necessary to perform a cleanup step to remove all *m*-CBA following oxidation of OTFPmOH to $OTFPmOHSO_2$. Roe et al. reported that they were able to wash away all of the acid byproduct with sodium bicarbonate (*sat*). We found that washing alone was not sufficient to remove all of the acid, unless an extremely large number of washes (>10) was used. Precipitation of *m*-CBA with sodium fluoride was a much more successful method. We also had to alter the DMP reaction conditions. In particular, Roe et al. used a reaction time of 3 h to oxidize $OTFPmOHSO_2$ to $OTFPdOHSO_2$. We found that 3 h was too long and that reaction times greater than 1 h resulted in decomposition

Table 2. Compounds Assayed for Esterase Inhibition Reported as IC_{50} (10^{-9} M)[a]



TFK



TFKdOHSO$_2$

	juvenile hormone esterase activity IC_{50}			rat carboxylesterase activity IC_{50}			porcine carboxylesterase activity IC_{50}		
R	TFK	TFKdOHSO$_2$	ratio TFKdOHSO$_2$/TFK	TFK	TFKdOHSO$_2$	ratio TFKdOHSO$_2$/TFK	TFK	TFKdOHSO$_2$	ratio TFKdOHSO$_2$/TFK
hexyl	31[a]	354 ± 20	11	54.6 ± 2.2	1290 ± 90	23	54.3 ± 6.3	39 000 ± 3900	719
octyl	1.6 ± 0.1	147 ± 89	92	37.2 ± 2.1	24.5 ± 3.7	0.66	36.8 ± 2.4	5640 ± 540	152
decyl	20[a]	119 ± 23	6	115 ± 10	6.3 ± 1.3	0.05	135 ± 8.0	1420 ± 53.0	11
dodecyl	15[a]	191 ± 9.0	13	164 ± 50	10.3 ± 2.6	0.06	119 ± 16	473 ± 26	4
phenethyl	ND[b]	1780 ± 570		162 ± 17	1500 ± 100	9	75.3 ± 5.6	3390 ± 210	52

[a] IC_{50} values taken from Szekacs et al. (*30*). [b] Not determined. Compounds are composed of two different schemes as shown below, trifluoromethyl ketone (TFK) or dihydroxysulfone trifluoromethyl ketone (TFKdOHSO$_2$), with a range of substituents (R).

of the molecule. It was necessary for Roe et al. to synthesize the DMP, since it was not commercially available at the time of their work. It is therefore possible that the DMP used by Roe et al. was of lower purity than that now available through T. C. I. America, which could account for the difference in reaction times. However, we successfully carried out the reaction with commercial as well as synthetic DMP.

The oxidation of the thioether in compound **2a** to the sulfone affected the hydration state of the ketone. The IR of **2a** showed stretching at 1750.3 cm^{-1}, typical for a polarized ketone, and there was no stretching in the 3000−3500 cm^{-1} range where a hydroxyl group would be expected to absorb. However, in compound **2b**, the IR showed no stretching in the ketone window and 2 distinct broad bands at 3439.4 and 3523.9 cm^{-1}. It is possible that the sulfone is responsible for shifting the *gem*-diol/ketone equilibrium such that the majority of the compound is hydrated. By ^{19}F NMR, this equilibrium was estimated to be >95% *gem*-diol in compound **2b** as compared to <9% *gem*-diol in compound **2a** (Table 1). This phenomenon could have a large effect upon inhibitor potency by favoring one hydration state. If the ketone is indeed the active form of the inhibitor, it will be necessary for the *gem*-diol compound to dehydrate back to the ketone before it binds to the nucleophilic serine residue. This dehydration step could occur either before the inhibitor enters the active site or after and could be the rate-limiting step of inhibition (*35*).

All analytical work conducted supported the structures as reported (Table 1). Of particular interest are the melting point data. Compounds **1b**, **2b**, and **5b** had sharp melting points, but compounds **3b** and **4b** were waxy solids with wide melting ranges. These compounds, however, showed >97% purity by GC/FID, TLC, ^{1}H NMR, and ^{19}F NMR and did not produce UV sensitive spots on F_{254} silica plates (thus indicating the absence of the *m*-CBA impurity). In addition, their mass spectral fragmentation patterns were consistent with other compounds in this series. It was therefore concluded that the longer chain compounds did not solidify at room temperature like the shorter chain compounds. This phenomenon could be due to the increased lipid-like character of these longer chain compounds. Also of interest was that the *gem*-diol compounds dehydrated during electrospray ionization mass spectrometry (ESI-MS) analysis. This observation further points to the dynamic nature of the keto/hydrate equilibrium.

(B) Enzyme Inhibition. We evaluated the inhibitory potency of these new compounds, reported as the IC_{50}, in three different enzyme systems. JHE activity was examined in order to compare results with data reported by Roe et al. (*35*), and rat and porcine carboxylesterases were used to examine inhibition potency in mammalian systems. Rat liver carboxylesterases have been extensively studied by several research groups (*7*, *19*) and crude porcine liver carboxylesterase is a good benchmark esterase due to its commercial availability (Sigma Chemical Co.). All enzyme activity data are presented in Table 2. Results are also expressed as the ratio of the IC_{50} of the sulfone analogue to the IC_{50} of the thioether compound for each enzyme. These ratios are not kinetic values and are only valid for comparisons among a given data set, but are useful for comparing relative differences in potency between sulfone and thioether compounds. It is also important to note that inhibitor potency can vary with the solvent used for the assay.

It is very likely that the ratio and sensitivity of individual enzyme isoforms varies greatly between the different species in this study, thus providing for different biochemical responses for each species. It is therefore important not to compare individual IC_{50} values for given compounds from each species. Instead only overall trends in rank potency should be compared. It is also important to remember that all enzyme preparations used in this study were crude mixtures potentially consisting of multiple esterase isoforms. We therefore refer to rat and porcine carboxylesterase as carboxylesterase activity due to the presence of a mixture of isozymes in the enzyme preparations. However, several laboratories demonstrated that *T. ni* hemolymph contains only one enzyme (JHE) that is responsible for JH hydrolysis (*49*, *50*). Additionally, JH is thought to be the natural substrate for JHE and is not significantly hydrolyzed by other enzymes in *T. ni* hemolymph (*50*), therefore, making it a good substrate for JHE activity assays. This exceptional specificity is thought to be due to the α,β-unsaturation of the methyl ester rendering it stable to both base hydrolysis and the action of most esterases (*51*). The crude porcine carboxylesterase purchased from Sigma is partially purified (*39*) as opposed to the rat liver microsomal preparation, which undoubtedly consist of multiple

enzymes and several different isoforms of carboxylesterase (*7*). The substrate PNPA, used for the mammalian carboxylesterase studies, is a ubiquitous nonselective substrate that is a very broad indicator of esterlytic activity. However, it is an excellent substrate for measuring carboxylesterase activity because all carboxylesterases identified to date are capable of hydrolyzing PNPA (*52*). In addition, Ashour and Hammock reported that PNPA is a good substrate for distinguishing between some protease and carboxylesterase activity (*39*), as carboxylesterases are 10^5 times more efficient at hydrolyzing PNPA than proteases such as chymotrypsin (*53*). Kao et al. (*54*) reported that Type A esterases, which hydrolyze organophosphates, did not hydrolyze PNPA, and Wallace et al. (*19*) found that inhibiting carboxylesterase activity in rat microsomes with paraoxon eliminated 100% of PNPA hydrolysis. PNPA was therefore chosen as an appropriate substrate for all mammalian carboxylesterase activity assays.

All sulfone compounds were consistently poorer inhibitors of JHE than their thioether analogues. Roe et al. reported that compound **2b** was three times more potent than **2a** against *T. ni* JHE (*35*). Our results showed an opposite effect with compound **2b** being almost 100-fold less potent than **2a**. The discrepancies in IC_{50} results could be due to differences in insect strains, enzyme preparation, solvent used for inhibitor dilutions, and assay conditions between the two laboratories. However, it was not possible to verify the enzyme preparation specifics from Roe et al. based upon published information. Compound **3b** was the most potent sulfone inhibitor of JHE with an IC_{50} of 119 ± 23 nM and had a sulfone to thioether IC_{50} ratio of six, indicating that the thioether compound (**3a**) was six times more potent than **3b**. This compound was still a relatively weak inhibitor compared to the most potent inhibitor of JHE, compound **2a** with an IC_{50} of 1.6 ± 0.1 nM. This value agrees well with previously published results (*27*) and further demonstrates that **2a** is one of the most potent JHE inhibitors synthesized to date.

The results with rat liver carboxylesterase activity were remarkably different from those of JHE, with three of the sulfone analogues (compounds **2b**, **3b**, and **4b**) being substantially more potent than their thioether analogues (compounds **2a**, **3a**, and **4a**). The decyl sulfone derivative, **3b**, proved to be the most potent mammalian inhibitor of all compounds tested with a sulfone to thioether IC_{50} ratio of 0.05 and an IC_{50} of 6.3 ± 1.3 nM (Table 2). Compound **3b** is 15-fold more potent than its thioether analogue and 6-fold more potent than the previous best inhibitor of rat carboxylesterase activity, compound **2a** with an IC_{50} of 37.2 ± 2.1 nM. Previous studies with TFK inhibitors have shown that long-chain aliphatic groups are the most potent carboxylesterase inhibitors (*55*). This trend holds true with the sulfone derivatives, with maximum inhibition potency reached with 10 carbons. The hexyl compound **1b** provided a very interesting result. The IC_{50} ratio of sulfone analogue to thioether compound was 23, the largest for any of the rat carboxylesterase activity inhibitors. The addition of a sulfone appears to interfere with the binding of the shorter-chain inhibitors. It is possible that the increased water solubility of **1b** overcomes the hydrophobicity of the shorter chain hexyl compound, whereas the longer chain inhibitors still have high enough log *P* values to effectively bind to the enzyme. The estimated log *P* value for **1b** is 1.17 versus 3.46 for **1a**, whereas the values for **3b** and **3a** are 3.28 and 5.57, respectively (Table 3). It is possible that there is a minimum threshold log *P* value for efficient binding of the inhibitor. Upon the basis of our inhibition results, this value is probably between 2 and 3. Therefore, it appears that compounds with longer aliphatic chains have hydrophobicities large enough to overcome the repulsion effects arising from the addition of the sulfone moiety. This result is in agreement with quantitative structure−activity relationship (QSAR) studies conducted by Székács et al. that determined that lipophilicity was the most important descriptor, being responsible for over 60% of the biological activity of the TFK inhibitors (*30*).

Table 3. Calculated log *P* Values for Synthesized Compounds

no.	ClogP[a]	no.	ClogP[a]
1a	3.46	**4a**	6.63
1b	1.17	**4b**	4.34
2a	4.51	**5a**	2.91
2b	2.23	**5b**	0.62
3a	5.57	**JH III**[b]	4.35
3b	3.28		

[a] ClogP indicates log *P* value calculated using the ClogP program developed by Leo et al. (*58*). [b] JH III = juvenile hormone III.

The crude porcine carboxylesterase exhibited a dramatically different response than that of the rat liver microsomal preparation for the sulfone derivatives, which were consistently worse inhibitors than the thioether analogues. Compound **1b** was particularly bad with a sulfone/thioether IC_{50} ratio of 719, the highest of any of the compounds and enzymes tested. However, the trends do mirror those seen for rat carboxylesterase activity, with potency increasing with the length of the aliphatic chain. Maximal inhibition for porcine carboxylesterase activity was achieved with a dodecyl group (**4b**), which had a sulfone to thioether IC_{50} ratio of four. The differences in activity between rat and porcine carboxylesterase activity probably result from differences in the physiochemical environment within the active site of the enzyme, with the porcine enzyme possibly requiring a more hydrophobic inhibitor. Further studies could compare the steric and electronic fields within the active sites of the two enzymes to explain the variations in activity. From our results, we can say that the enzyme pocket appears to have a hydrophobic character that favors inhibitors and substrates with high log *P* values. It is also interesting that the results for the thioether compounds for the rat and porcine enzymes are remarkably similar. All of the aliphatic compounds have essentially the same IC_{50} value within the accuracy of the assays. This result also points to the importance of log *P* in the inhibition of these enzymes. Compounds with a given log *P* value inhibit carboxylesterases from different mammalian species with nearly identical potency.

The reasons for the sulfone analogues being consistently less potent inhibitors of *T. ni* JHE and porcine carboxylesterase activity than their thioether analogues are unclear. Without structural evidence, such as a crystal structure of both enzymes, it is difficult to compare catalytic mechanisms. There is no structural information available for *T. ni* JHE, but Ward et al. showed that the JHE of *Heliothis virescens* contains a catalytic triad comparable to other esterases (Ser-Arg-

Glu) (*56*). It is possible that the sulfone is engaging in unfavorable secondary interactions within the JHE active site of *T. ni*. JHE is highly selective for JH-like molecules, and even though its catalytic triad is similar in composition to that of the rat carboxylesterase (Ser-His-Glu) (*7*), the overall sequences show very little homology. Given the selectivity of JHE for methyl esters, it would be expected that any bulky substituent that interfered with the placement of the substrate or inhibitor within the correct distance of the nucleophilic serine could decrease activity. Thus, it is possible that the relatively large bulk of the sulfone, compared to the thioether, sterically hinders the binding of the inhibitor to the active residue, resulting in lower potency. However, Linderman et al. showed that compounds containing a methyl group either α or α′ to the thioether were still very potent inhibitors of JHE (*29*). Therefore, the reduction in potency of the sulfone compounds is probably electronically mediated. There could potentially be electronic repulsion resulting from the high electron density of the oxygens of the sulfone moiety. An additional possibility is that the active site of JHE is very hydrophobic, resulting in unfavorable interactions with compounds having low log *P* values. It would be necessary to further analyze the position of these inhibitors within the active site of JHE to accurately predict their potential interactions with all residues in the active site.

There are several possibilities that may explain the increased potency of the sulfone analogue in the rat liver microsomal preparation. First, it is possible that the sulfone is hydrogen bonding with a hydroxy group on the *gem*-diol. Although thioethers normally form weak hydrogen bonds, crystallographic analysis of 1,1,1-trifluoro-3-phenethylsulfanyl-propan-2-one showed that the structure was hydrated and that a tight hydrogen bond formed between the sulfur in the thioether and one of the hydroxyl groups in the *gem*-diol, forming a five-membered ring (*57*). It is probable that similar intramolecular hydrogen bonding is also occurring in the sulfone compound. It is therefore possible that the six-membered ring of the sulfone compound is energetically favored over that of the five-membered thioether compound, thus accounting for the difference in inhibitor potencies. Following nucleophilic attack by serine, the enzyme/inhibitor complex would resemble the transition state of the enzyme with its natural substrate, forming a tetrahedral intermediate. Formation of a six-membered ring would stabilize this intermediate, thus preventing cleavage of the oxygen−carbon bond between the serine and the inhibitor (Figure 1). This mechanism would result in a more potent inhibitor. Also possible is that the sulfone, given its electron-withdrawing nature, increases the positive polarization of the carbonyl carbon and/or shifts the *gem*-diol/ketone equilibrium toward the diol, thereby stabilizing the product of a nucleophilic attack on the ketone form of the sulfone TFKs.

Solubility studies with **2a** and **2b** demonstrated that the oxidation of the thioether to the sulfone increases water solubility. The water solubility of **2a** was between 0.2 and 0.3 mM whereas the solubility of **2b** was between 1.5 and 2.0 mM. Compound **2b** is therefore approximately 10 times more soluble under the conditions examined (Figure 2). An inhibitor that is more potent and water-soluble would be very useful for applications in affinity chromatography. One difficulty often encountered in affinity purification of esterases with TFK inhibitors is

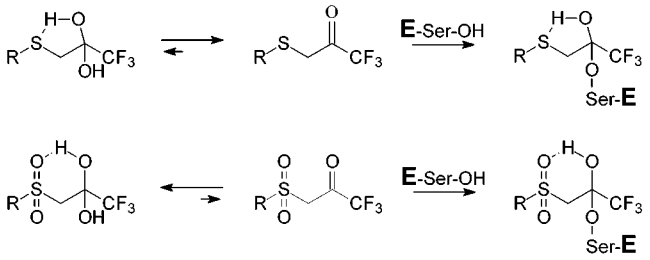


Figure 1. Proposed mechanism for intramolecular hydrogen bond formation for sulfone and thioether trifluoromethyl ketones (TFKs). Graphic shows the dynamic equilibrium between the ketone and hydrate forms of the TFKs. The ketone is considered to be the active form of the inhibitor, which undergoes nucleophilic attack by the hydroxy moiety of the esterase (**E**) catalytic serine residue (Ser-OH). Hydrogen bonds are shown as a dashed line. Crystallographic data showed the formation of a hydrogen bond between the sulfur and hydrated ketone in 1,1,1-trifluoro-3-phenethylsulfanyl-propan-2-one (*57*).

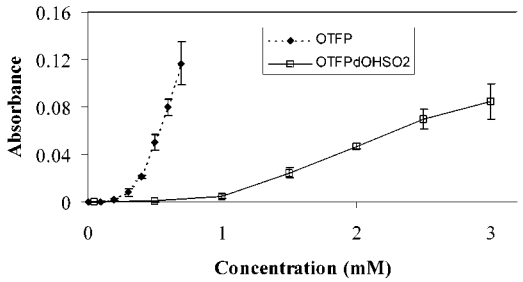


Figure 2. Solubility determination of 1,1,1-trifluoro-3-(octane-1-sulfonyl)-propan-2,2-diol (OTFPdOHSO$_2$, **2b**) and 1,1,1-trifluoro-3-octylsulfanyl-propan-2-one (OTFP, **2a**). Compounds were dissolved in dimethylformamide and 10 μL of solution was added to 1 mL of sodium phosphate buffer (pH 7.4, 0.1 M) in a cuvette at 25 °C. The turbidity of the solution was then measured by reading the absorbance at 700 nm. Compound solubility was determined by a significant increase in absorption (>4-fold) over baseline values ($n = 3$).

the regeneration of catalytically active enzyme (*27*, *55*). Enzyme eluted from an affinity gel is often still inhibited and remains so even after extensive dialysis. The use of more potent inhibitors with increased water solubility could greatly assist in the diffusion of the inhibitors out of the active site of the enzyme and through the dialysis membrane, regenerating catalytically active enzyme. It may is also be possible to use higher concentrations of weaker inhibitors to elute the enzymes from the affinity column.

Carboxylesterases are important in the hydrolysis and metabolism of numerous ester-containing compounds. Given their role in the activation of prodrugs, metabolism of pharmaceuticals and xenobiotics, and detoxification of pesticides, it is essential that we understand the mechanism by which these enzymes function. Toward this end we have synthesized four new inhibitors that will provide tools to explore key differences in the catalytic mechanism of different carboxylesterases. These new inhibitors have ortholog specific potency and could prove useful for distinguishing specific esterase isoforms. These compounds could also assist in the affinity purification of these essential enzymes. Further research should address the mechanism behind the variation in potency of these different compounds. Analysis of the key physiochemical parameters responsible for carboxylesterase activity will greatly increase our understanding of these enzymes.

Acknowledgment. The authors gratefully acknowledge Dr. Christophe Morisseau and Dr. Donald Stoutamire from the Department of Entomology at the University of California Davis for many useful discussions. We also thank Dr. Yoshiaki Nakagawa and Dr. Masahiro Miyashita from the Graduate School of Agriculture at Kyoto University for performing the log *P* calculations. C.E.W. was supported by a UC TSR&TP Lead Campus Program in Ecotoxicology Fellowship and a UC TSR&TP Graduate Fellowship. T.F.S. was supported by USDA Competitive Research Grant 2001-35302-09919 and a UC Davis Biotechnology Training Grant Fellowship. This work was supported in part by NIEHS Grant R37 ES02710, NIEHS Superfund Grant P42 ES04699, and NIEHS Center for Environmental Health Sciences Grant P30 ES05707.

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