Synthesis of *N*-(Trifluoromethyl-2-pyridinyl)arenesulfonamides as an Inhibitor of Secretory Phospholipase A_2

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A series of *N*-(trifluoromethyl-2-pyridinyl)alkane- and arenesulfonamides 2—5 have been synthesized by the substitution reaction of 2-chloro(trifluoromethyl)pyridines 6 with alkane- and arenesulfonamides 7. Their inhibitory activities against secretory phospholipase A_2 of porcine pancreas were examined and the analog *N*-[4,5-bis-(trifluoromethyl)-2-pyridinyl]-4-trifluoromethylbenzenesulfonamide 4i was shown to have the highest inhibitory activity, with an IC_{50} value of 0.58 mM.

Key words bistrifluoromethylpyridine; sulfonamide; secretory phospholipase A_2 inhibitor; structure–activity relationship

Phospholipase A_2 (PLA_2) is an enzyme that catalyzes the hydrolysis of membrane glycerophospholipids at the 2 position to produce free fatty acids, in particular arachidonic acid, and lysophospholipids. Among a number of PLA_2 isozymes identified so far, several isozymes including secretory forms are involved in the release of arachidonic acid, a precursor for prostaglandins and leukotrienes.[1] Since the eicosanoids are potent mediators of inflammation, the inhibition of PLA_2 is a prime target for designing new anti-inflammatory agents.[2—7] In fact, a number of its inhibitors such as varespladib,[8] pyrrophenone,[9] efipladib[10] and methyl indoxam[11] have been reported. *N*-[2-(Ethanesulfonylamino)-5-trifluoromethyl-3-pyridinyl]cycloalkanecarboxamide (**1**) having a trifluoromethyl (CF_3)-pyridine ring was reported to possess a moderate inhibitory activity against pancreatic secretory phospholipase A_2 ($sPLA_2$) in *in vitro* experiments.[12] Trifluoromethyl-substituted pyridines are useful ring unit[13,14] and have shown interesting profiles as essential structures in many kinds of biological molecules including agrochemical and medicinal candidates.[15—18] We have re-investigated the above molecule and attempted to simplify and modify its structure in order to improve the inhibitory activity for PLA_2 (Fig. 1).

We have designed new molecules by the following directions; i) removal of the 3-cycloalkanecarboxamide moiety from **1**, ii) change of the position of the CF_3 group and introduction of an additional CF_3 group or other electron withdrawing group, iii) replacement of ethanesulfonamide with other alkane- and arenesulfonamides. In this paper, we describe the preparation of compounds **2**—**5**, designed by the above direction, and evaluation of their structure–activity relationships with respect to the inhibiting activity against PLA_2 Group IB (GIB).

The structure of designed compounds are listed in Fig. 2. We categorized the analogs by 5-trifluoromethyl, 4-trifluoromethyl, 4,5-bis(trifluoromethyl), and 4,6-bis(trifluoromethyl) groups, as compounds, **2a**—**d**, **3a**—**d**, **4a**—**k**, and **5a**—**f**, respectively.

The formation of the amide bond was carried out simply by heating of 2-chloropyridine **6a**—**f** and alkane- or arenesulfonamide **7a**—**j** with K_2CO_3 in dimethyl sulfoxide (DMSO) at 115—150 °C. For example, the reaction of 2-chloro-5-trifluoromethylpyridine (**6a**) and ethanesulfonamide (**7a**) (1.5 eq) in DMSO in the presence of K_2CO_3 (2.5 eq) at 130 °C for 2 h gave *N*-(5-trifluoromethyl-2-pyridinyl)ethanesulfonamide (**2a**) in 65% yield. Other molecules, with the exception of **4k**, were similarly prepared and the results are as shown in Table 1. The compound **4k** was prepared in 85% yield from **3a** by the standard nitration.

All new sulfonamides were evaluated for their inhibiting activity against porcine pancreas originated $sPLA_2$. The activity was assessed by measuring free fatty acids released from phospholipids, as a substrate, with photometric assay using the acyl-CoA synthetase/acyl-CoA oxidase (ACS–ACO) method.[19] We have screened all the sulfonamides at a fixed concentration (0.5 mM), and some of them were also

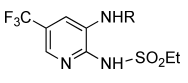
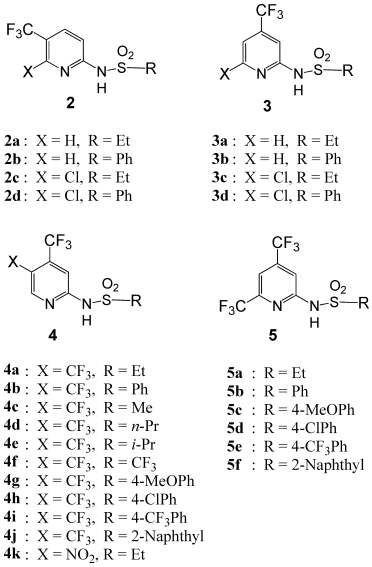


Fig. 1. Structure of **1** (R=Cycloalkanecarbonyl)



2a : X = H, R = Et
2b : X = H, R = Ph
2c : X = Cl, R = Et
2d : X = Cl, R = Ph

3a : X = H, R = Et
3b : X = H, R = Ph
3c : X = Cl, R = Et
3d : X = Cl, R = Ph

4a : X = CF_3, R = Et
4b : X = CF_3, R = Ph
4c : X = CF_3, R = Me
4d : X = CF_3, R = *n*-Pr
4e : X = CF_3, R = *i*-Pr
4f : X = CF_3, R = CF_3
4g : X = CF_3, R = 4-MeOPh
4h : X = CF_3, R = 4-ClPh
4i : X = CF_3, R = 4-CF_3Ph
4j : X = CF_3, R = 2-Naphthyl
4k : X = NO_2, R = Et

5a : R = Et
5b : R = Ph
5c : R = 4-MeOPh
5d : R = 4-ClPh
5e : R = 4-CF_3Ph
5f : R = 2-Naphthyl

Fig. 2. Structures of Compounds **2**—**5**

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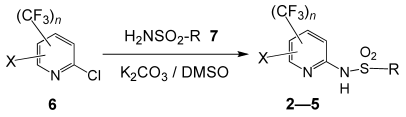
screened at 3 mM concentration. IC_{50} values were determined for four compounds. These results are summarized in Table 1.

If a functional substituent on the pyridine ring was replaced with hydrogen without loss of biological activity, it would reduce the number of synthetic steps. In fact, for the synthesis of compounds **2**—**5**, the synthesis would become much simpler in comparison with that of **1**. Replacing the C-3 carboxamide moiety in **1** with hydrogen afforded 5-trifluoromethyl substituted ethanesulfonamide **2a** (entry 1), which inhibited $sPLA_2$ by 4.6%. Compound **3a** in which a CF_3 group located at the C-4 position instead of at C-5, inhibited by 20.6%. In comparison of compounds **2a** and **3a** (entries 1 and 5), in both of which the C-3 position is unsubstituted, the inhibitory activity of **3a** exceeded that of **2a**, indicating the importance of the CF_3 substituent at the C-4 site. When ethanesulfonamides in **2a** and **3a** were replaced with benzenesulfonamides gave **2b** and **3b**, the inhibitory activity enhanced to 15.4% and 25.0% in both cases (entries 2 and 6). The presence of a chloro group at the C-6 position, compounds **2c** and **2d** (entries 3 and 4) provided inhibitors that were slightly active than compounds **2a** and **2b**. While, the corresponding 4-trifluoromethyl derivatives **3c** and **3d** (entries 7 and 8) were less active than **3a** and **3b**. It is noted that benzenesulfonamides **2b**, **2d**, **3b** and **3d** were more potent than ethanesulfonamides **2a**, **2c**, **3a** and **3c**, respectively.



6a : X = H, n = 1, 5-CF_3
6b : X = 6-Cl, n = 1, 5-CF_3
6c : X = H, n = 1, 4-CF_3
6d : X = 6-Cl, n = 1, 4-CF_3
6e : X = H, n = 2, 4,5-$(CF_3)_2$
6f : X = H, n = 2, 4,6-$(CF_3)_2$

7a : R = Et
7b : R = Ph
7c : R = 4-MeOPh
7d : R = 4-ClPh
7e : R = Me
7f : R = *n*-Pr
7g : R = *i*-Pr
7h : R = CF_3
7i : R = 4-CF_3Ph
7j : R = 2-Naphthyl

Chart 1. Synthesis of Sulfonamides **2**—**5**

Since the CF_3 substituent at C-4 showed a better inhibitory profile than that at C-5, we examined 4,5-bis(trifluoromethyl)pyridine derivatives **4**. In the cases of **2a**—**d** and **3a**—**d**, benzenesulfonamides were more potent than ethanesulfonamides. A little but similar trend was observed in 4,5-bis(triflluoromethyl)pyridine derivatives **4a** and **4b** (entries 9 and 10). *N*-[4,5-Bis(trifluoromethyl)-2-pyridinyl]ethanesulfonamide (**4a**) and the corresponding benzenesulfonamide **4b**, each at 0.5 mM inhibited PLA_2GIB by 24.5% and 26.4%, respectively. Other alkanesulfonamides, the respective methyl-, propyl-, and isopropyl analogs, **4c**, **4d**, and **4e**, as well as trifluoromethanesulfonamide **4f** (entries 11—14), all showed inferior activity than **4a**.

Next, we examined the effect of the substituent on the aromatic ring of the arenesulfonamide in the series of *N*-[4,5-bis(trifluoromethyl)-2-pyridinyl] derivatives. 4-Methoxybenzenesulfonamide **4g** (entry 15) was slightly active to compare with the phenyl analogue **4b**, while the 4-chloro-, and 4-trifluoromethylbenzenesulfonamides **4h** and **4i** (entries 16 and 17) were 1.4 fold more active than **4b**. 2-Naphtharenesulfonamide **4j** (entry 18) was a little less potent than **4h** and **4i**. Replacement of an electron-withdrawing CF_3 group at the C-5 position with other electron-withdrawing nitro group, in **4k** (entry 19), decreased the inhibitory potency by 25%, in comparison to **4a**. These results indicated that the 4,5-bis(trifluoromethyl) substituent pattern positively influenced the inhibitory activity.

Table 1. Reaction Conditions, Yields and $sPLA_2$-Inhibiting Activities of *N*-(Trifluoromethyl-2-pyridinyl)alkane- and Arenesulfonamides **2**—**5**

Entry	Chloro-pyridine **6**	Sulfon-amide **7**	Product	Yield[a] (%)	Position of $(CF_3)_n$	X	R	Inhibition (%)		IC_{50} (mM)
								at 0.5 mM	at 3 mM	
1	**6a**	**7a**	**2a**	65	5-	H	Et	4.6		
2	**6a**	**7b**	**2b**	43	5-	H	Ph	15.4		
3	**6b**	**7a**	**2c**	56	5-	6-Cl	Et	15.4		
4	**6b**	**7b**	**2d**	70	5-	6-Cl	Ph	25.6	85.3	1.0
5	**6c**	**7a**	**3a**	37	4-	H	Et	20.6		
6	**6c**	**7b**	**3b**	71	4-	H	Ph	25.0		
7	**6d**	**7a**	**3c**	76	4-	6-Cl	Et	8.9		
8	**6d**	**7b**	**3d**	32	4-	6-Cl	Ph	16.1		
9	**6e**	**7a**	**4a**	83	4,5-	H	Et	24.5		
10	**6e**	**7b**	**4b**	92	4,5-	H	Ph	26.4	87.5	
11	**6e**	**7e**	**4c**	93	4,5-	H	Me	16.5		
12	**6e**	**7f**	**4d**	71	4,5-	H	*n*-Pr	4.3		
13	**6e**	**7g**	**4e**	89	4,5-	H	*i*-Pr	3.1		
14	**6e**	**7h**	**4f**	50	4,5-	H	CF3	13.3		
15	**6e**	**7c**	**4g**	53	4,5-	H	4-MeOPh	28.4		
16	**6e**	**7d**	**4h**	46	4,5-	H	4-ClPh	36.7	89.6	0.66
17	**6e**	**7i**	**4i**	68	4,5-	H	4-CF3Ph	36.1	98.3	0.58
18	**6e**	**7j**	**4j**	61	4,5-	H	2-Naphthyl	30.8	85.0	
19	—	—	**4k**	—	4-	5-NO_2	Et	18.0		
20	**6f**	**7a**	**5a**	81	4,6-	H	Et	23.0		
21	**6f**	**7b**	**5b**	75	4,6-	H	Ph	25.6	91.1	
22	**6f**	**7c**	**5c**	87	4,6-	H	4-MeOPh	20.0		
23	**6f**	**7d**	**5d**	90	4,6-	H	4-ClPh	14.4		
24	**6f**	**7i**	**5e**	81	4,6-	H	4-CF_3Ph	28.1	83.2	
25	**6f**	**7j**	**5f**	75	4,6-	H	2-Naphthyl	34.6	91.2	0.68

a) Isolated yields. The yields were not optimized.

We have also examined 4,6-bis(trifluoromethyl)pyridine derivatives **5**. Ethane- and benzenesulfonamides **5a** and **5b** (entries 20 and 21) showed similar potency as same as **4a** and **4b**. 4-Methoxy- and 4-trifluoromethylbenzenesulfonamides **5c** and **5e** (entries 22 and 24) were less active than the corresponding **4g** and **4i**. However, somehow the 4-chlorobenzenesulfonamide **5d** (entry 23) was 1.8-fold less active than **5b**. 2-Naphtharenesulfonamide **5f** (entry 25) was the most active of the 4,6-bis(trifluoromethyl)pyridine derivatives. Comparison of the inhibition data between 5-CF_3, 6-CF_3, 4,5-bis-CF_3 and 4,6-bis-CF_3 derivatives indicate that the 4,5-bis(trifluoromethyl) as well as 4,6-bis(trifluoromethyl)substituent patterns effectively show better inhibitory activities.

Inhibitory activities of several selected compounds were tested at the 3 mM concentration. These results were consistently supported the results obtained at the 0.5 mM concentration. The IC_{50} values for compounds **2d**, **4h**, **4i**, and **5f** are listed in Table 1. Particularly, *N*-[4,5-bis(trifluoromethyl)-2-pyridinyl]-4-trifluoromethylbenzenesulfonamide **4i** exhibited the most potent IC_{50} value (at 0.58 mM) in the inhibition of $sPLA_2$. Although they are lacking a carboxamide moiety at the C-3 position on the pyridine ring, their activities are almost similar or even better than those of the compounds **1**.[12)]

In conclusion, we have demonstrated a simple CF_3-substituted 2-pyridinylarenesulfonamides exhibited an inhibitory activity of $sPLA_2$. Although their acivitiy is moderate, *N*-[bis(trifluoromethyl)-2-pyridinyl]arenesulfonamides **4h**, **4i**, and **5f** showed excellent profile in the inhibition. Particularly, compound **4i** was found to be the most potent. It is noted that all these compounds are able to prepare quite easily by a single coupling of **6** with sulfonamide **7**.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and were not corrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-GSX 400 (400 MHz) or JNM-AL 300 (300 MHz) spectrometers in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as an internal standard. IR spectra were recorded on JASCO FT/IR-410 instrument. Column chromatography was carried out using Merck silica gel 60 (70—230 mesh).

Typical Reaction for Coupling of 2-Chloropyridines 6a—f and Sulfonamides 7a—j To a mixture of **7** (7.5 mmol) and K_2CO_3 (1.73 g, 12.5 mmol) in dimethyl sulfoxide (DMSO) (20 ml) was added **6** (5 mmol) in DMSO (2 ml) portionwise at 95 °C. The mixture was heated at 115—150 °C for 1.5—17 h. After cooling, water (50—100 ml) was added to the reaction mixture, and the mixture was washed with ether. The pH of the aqueous layer was adjusted to a range between 4 to 6 with 2 N hydrochloric acid to form a precipitate, which was collected and recrystallized from a mixture of EtOAc and hexane to give **2**—**5**. When precipitate was not formed, the aqueous layer was extracted with EtOAc (50 ml×2), the combined organic layer was washed with brine, dried over Na_2SO_4, and condensed. The residual liquid was purified by silica gel column chromatography. For the synthesis of **3c** and **3d**, reverse addition was taken.

Chemical yields of the products are listed in Table 1.

***N*-(5-Trifluoromethyl-2-pyridinyl)ethanesulfonamide (2a)** Reaction condition, 130 °C, 2 h. Pale yellow powder; mp 164—166 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, t, J=7.4 Hz), 3.35 (2H, q, J=7.4 Hz), 7.45 (1H, d, J=8.8 Hz), 7.93 (1H, dd, J=8.8, 2.4 Hz), 8.67 (1H, s), 9.56 (1H, bs). IR (KBr) cm^{-1}: 1335, 1132. *Anal.* Calcd for $C_8H_9F_3N_2O_2S$: C, 37.79; H, 3.57; N, 11.02. Found: C, 37.88; H, 3.50; N, 10.99.

***N*-(5-Trifluoromethyl-2-pyridinyl)benzenesulfonamide (2b)** Reaction condition, 130 °C, 3 h. Pale yellow solid; mp 161—163 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.47—7.54 (3H, m), 7.61 (1H, m), 7.85—7.91 (3H, m), 8.74 (1H, s), 11.36 (1H, bs). IR (KBr) cm^{-1}: 1328, 1167. *Anal.* Calcd for $C_{12}H_9F_3N_2O_2S$: C, 47.68; H, 3.00; N, 9.27. Found: C, 47.71; H, 2.78; N, 9.18.

***N*-(6-Chloro-5-trifluoromethyl-2-pyridinyl)ethanesulfonamide (2c)** Reaction condition, 115 °C, 17 h. White prisms; mp 105—107 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (3H, t, J=7.5 Hz), 3.75 (2H, q, J=7.5 Hz), 7.14 (1H, bs), 7.15 (1H, dd, J=8.1, 0.75 Hz), 7.85 (1H, dd, J=8.1, 0.6 Hz). IR (KBr) cm^{-1}: 1345, 1160. *Anal.* Calcd for $C_8H_8ClF_3N_2O_2S$: C, 33.29; H, 2.79; N, 9.70. Found: C, 33.36; H, 2.53; N, 9.75.

***N*-(6-Chloro-5-trifluoromethyl-2-pyridinyl)benzenesulfonamide (2d)** Reaction condition, 120 °C, 3.5 h. Pale yellow solid; mp 104—105 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.32 (1H, d, J=8.4 Hz), 7.51—7.57 (2H, m), 7.64 (1H, m), 7.83 (1H, bs), 7.90 (1H, d, J=8.4 Hz), 7.96—7.99 (2H, m). IR (KBr) cm^{-1}: 1313, 1146. *Anal.* Calcd for $C_{12}H_7Cl_2F_3N_2O_2S$: C, 38.83; H, 1.90; N, 7.55. Found: C, 38.70; H, 1.95; N, 7.50.

***N*-(4-Trifluoromethyl-2-pyridinyl)ethanesulfonamide (3a)** Reaction condition, 120 °C, 4 h. Pale yellow solid; mp 140.5—141 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7.5 Hz), 3.31 (2H, q, J=7.5 Hz), 7.27 (1H, d, J=4.8 Hz), 7.63 (1H, s), 8.63 (1H, d, J=4.8Hz). IR (KBr) cm^{-1}: 1335, 1145. *Anal.* Calcd for $C_8H_9F_3N_2O_2S$: C, 37.79; H, 3.57; N, 11.02. Found: C, 37.51; H, 3.54; N, 10.86.

***N*-(4-Trifluoromethyl-2-pyridinyl)benzenesulfonamide (3b)** Reaction condition, 140 °C, 3 h. White prisms; mp 139—140 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.21 (1H, d, J=5.4 Hz), 7.45—7.50 (2H, m), 7.59 (1H, m), 7.67 (1H, s), 7.85—7.88 (2H, m), 8.62 (1H, d, J=5.4 Hz), 10.82 (1H, bs). IR (KBr) cm^{-1}: 1335, 1171. *Anal.* Calcd for $C_{12}H_9F_3N_2O_2S$: C, 47.68; H, 3.00; N, 9.27. Found: C, 47.41; H, 2.97; N, 9.07.

***N*-(6-Chloro-4-trifluoromethyl-2-pyridinyl)ethanesulfonamide (3c)** Reaction condition, 120 °C, 1.5 h. White solid; mp 100—101 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (3H, t, J=7.3 Hz), 3.41 (2H, q, J=7.3 Hz), 7.27 (1H, s), 7.37 (1H, s), 7.65 (1H, bs). IR (KBr) cm^{-1}: 1316, 1147. *Anal.* Calcd for $C_8H_9F_3N_2O_2S$: C, 33.29; H, 2.79; N, 9.70. Found: C, 33.36; H, 2.74; N, 9.69.

***N*-(6-Chloro-4-trifluoromethyl-2-pyridinyl)benzenesulfonamide (3d)** Reaction condition, 130 °C, 2.5 h. White prisms; mp 138—139 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.20 (1H, s), 7.47 (1H, s), 7.51—7.57 (2H, m), 7.63 (1H, m), 7.75 (1H, bs), 7.95—7.97 (2H, m). IR (KBr) cm^{-1}: 1338, 1170, 687. *Anal.* Calcd for $C_{12}H_8ClF_3N_2O_2S$: C, 42.80; H, 2.39; N, 8.32. Found: C, 43.00; H, 2.20; N, 8.32.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]ethanesulfonamide (4a)** Reaction condition, 120 °C, 1.5 h. Pale yellow prisms; mp 174—175 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, t, J=7.3 Hz), 3.46 (2H, q, J=7.3 Hz), 7.55 (1H, s), 7.74 (1H, bs), 8.77 (1H, s). IR (KBr) cm^{-1}: 1321, 1146. *Anal.* Calcd for $C_9H_8F_6N_2O_2S$: C, 33.55; H, 2.50; N, 8.69. Found: C, 33.81; H, 2.47; N, 8.57.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]benzenesulfonamide (4b)** Reaction condition, 120 °C, 1.5 h. White prisms; mp 164—165 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.53—7.58 (2H, m), 7.64 (1H, m), 7.68 (1H, s), 7.96—7.98 (2H, m), 8.15 (1H, bs), 8.68 (1H, s). IR (KBr) cm^{-1}: 1326, 1142. *Anal.* Calcd for $C_{13}H_8F_6N_2O_2S$: C, 42.17; H, 2.18; N, 7.57. Found: C, 42.31; H, 2.07; N, 7.55.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]methanesulfonamide (4c)** Reaction condition, 130 °C, 3 h. Pale yellow needles; mp 163—164 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.35 (3H, s), 7.26 (1H, s), 7.51 (1H, s), 8.81 (1H, s). IR (KBr) cm^{-1}: 1322, 1153. *Anal.* Calcd for $C_8H_6F_6N_2O_2S$: C, 31.18; H, 1.96; N, 9.09. Found: C, 30.99; H, 1.87; N, 9.16.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]propanesulfonamide (4d)** Reaction condition, 120 °C, 2 h. Pale yellow needles; mp 145—146 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, t, J=7.4 Hz), 1.89—1.99 (2H, m), 3.39 (2H, t, J=7.9 Hz), 7.57 (1H, s), 7.96 (1H, bs), 8.79 (1H, s). IR (KBr) cm^{-1}: 1321, 1146. *Anal.* Calcd for $C_{10}H_{10}F_6N_2O_2S$: C, 35.72; H, 3.00; N, 8.33. Found: C, 35.96; H, 2.77; N, 8.38.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]isopropanesulfonamide (4e)** Reaction condition, 150 °C, 2 h. Pale yellow needles; mp 167—168 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (6H, d, J=6.8 Hz), 3.63 (1H, m), 7.67 (1H, s), 8.15 (1H, bs), 8.77 (1H, s). IR (KBr) cm^{-1}: 1325, 1280, 1138. *Anal.* Calcd for $C_{10}H_{10}F_6N_2O_2S$: C, 35.72; H, 3.00; N, 8.33. Found: C, 35.79; H, 2.78; N, 8.57.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]trifluoromethanesulfonamide (4f)** Reaction condition, 150 °C, 2 h. White prisms; mp 173—174 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.98 (1H, d, J=3.6 Hz), 8.26 (1H, s). IR (KBr) cm^{-1}: 1341, 1203. *Anal.* Calcd for $C_8H_3F_9N_2O_2S$: C, 26.53; H, 0.83; N, 7.73. Found: C, 26.79; H, 0.64; N, 7.90.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]-4′-methoxybenzenesulfonamide (4g)** Reaction condition, 120 °C, 2 h. Pale yellow prisms; mp 156—157 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (3H, s), 6.99 (2H, d, J=9.2 Hz), 7.64 (1H, s), 7.91 (2H, d, J=9.2Hz), 8.65 (1H, s). IR (KBr) cm^{-1}: 1328, 1156. *Anal.* Calcd for $C_{14}H_{10}F_6N_2O_3S$: C, 42.01; H, 2.52; N, 7.00. Found: C, 41.85; H, 2.45; N, 6.92.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]-4′-chlorobenzenesulfonamide (4h)** Reaction condition, 120 °C, 1.5 h. Pale yellow needles; mp 147—148 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.53 (2H, d, J=8.6 Hz), 7.61 (1H, s), 7.93 (2H,

d, J=8.6 Hz), 8.66 (1H, s). IR (KBr) cm^{-1}: 1328, 1159. *Anal.* Calcd for $C_{13}H_7ClF_6N_2O_2S$: C, 38.58; H, 1.74; N, 6.92. Found: C, 38.39; H, 1.76; N, 6.82.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]-4′-trifluoromethylbenzenesulfonamide (4i)** Reaction condition, 120 °C, 3 h. Yellow powder; mp 187—188 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.60 (1H, s), 7.82 (2H, d, J=8.2 Hz), 8.12 (2H, d, J=8.2 Hz), 8.67 (1H, s). IR (KBr) cm^{-1}: 1325, 1155. *Anal.* Calcd for $C_{14}H_7F_9N_2O_2S$: C, 38.37; H, 1.61; N, 6.39. Found: C, 38.09; H, 1.41; N, 6.40.

***N*-[4,5-(Bistrifluoromethyl)-2-pyridinyl]-2′-naphthalenesulfonamide (4j)** Reaction condition, 120 °C, 2 h. Pale yellow prisms; mp 193—194 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.60—7.74 (2H, m), 7.74 (1H, s), 7.85—7.95 (2H, m), 7.97—8.00 (2H, m), 8.58 (1H, s), 8.63 (1H, s), 10.2 (1H, bs). IR (KBr) cm^{-1}: 1324, 1160. *Anal.* Calcd for $C_{17}H_{10}F_6N_2O_2S$: C, 48.58; H, 2.40; N, 6.66. Found: C, 48.65; H, 2.39; N, 6.67.

***N*-[4,6-(Bistrifluoromethyl)-2-pyridinyl]ethanesulfonamide (5a)** Reaction condition, 115 °C, 3 h. White prisms; mp 91—93 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (3H, t, J=7.5 Hz), 3.51 (2H, q, J=7.5 Hz), 7.59 (2H, s), 7.90 (1H, bs). IR (KBr) cm^{-1}: 1334, 1140. *Anal.* Calcd for $C_9H_8F_6N_2O_2S$: C, 33.55; H, 2.50; N, 8.69. Found: C, 33.81; H, 2.47; N, 8.77.

***N*-[4,6-(Bistrifluoromethyl)-2-pyridinyl]benzenesulfonamide (5b)** Reaction condition, 120 °C, 3 h. White solid; mp 99—100 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.54 (1H, m), 7.51 (1H, s), 7.54 (1H, s), 7.61—7.66 (2H, m), 8.01—8.04 (2H, m). IR (KBr) cm^{-1}: 1336, 1166. *Anal.* Calcd for $C_{13}H_8F_6N_2O_2S$: C, 42.17; H, 2.18; N, 7.57. Found: C, 42.25; H, 1.96; N, 7.87.

***N*-[4,6-(Bistrifluoromethyl)-2-pyridinyl]-4′-methoxybenzenesulfonamide (5c)** Reaction condition, 140 °C, 2 h. White needles; mp 127—128 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.83 (3H, s), 7.12 (2H, d, J=8.7 Hz), 7.43 (1H, s), 7.83 (1H, s), 7.95 (2H, d, J=8.7 Hz), 11.96 (1H, bs). IR (KBr) cm^{-1}: 1317, 1185. *Anal.* Calcd for $C_{14}H_{10}F_6N_2O_3S$: C, 42.01; H, 2.52; N, 7.00. Found: C, 42.04; H, 2.61; N, 7.02.

***N*-[4,6-(Bistrifluoromethyl)-2-pyridinyl]-4′-chlorobenzenesulfonamide (5d)** Reaction condition, 120 °C, 3.5 h. White needles; mp 136—138 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.51 (2H, d, J=6.8 Hz), 7.53 (1H, s), 7.57 (1H, s), 7.96 (2H, d, J=6.8 Hz). IR (KBr) cm^{-1}: 1345, 1155. *Anal.* Calcd for $C_{13}H_7ClF_6N_2O_2S$: C, 38.58; H, 1.74; N, 6.92. Found: C, 38.62; H, 1.72; N, 7.01.

***N*-[4,6-(Bistrifluoromethyl)-2-pyridinyl]-4′-trifluoromethylbenzenesulfonamide (5e)** Reaction condition, 140 °C, 2 h. White needles; mp 158—159 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.56 (1H, s), 7.61 (1H, s), 7.81 (2H, d, J=8.1 Hz), 7.92 (1H, bs), 8.16 (2H, d, J=8.1 Hz). IR (KBr) cm^{-1}: 1420, 1330, 1170. *Anal.* Calcd for $C_{14}H_7F_9N_2O_2S$: C, 38.37; H, 1.61; N, 6.39. Found: C, 38.42; H, 1.75; N, 6.37.

***N*-[4,6-(Bistrifluoromethyl)-2-pyridinyl]-2′-naphthalenesulfonamide (5f)** Reaction condition, 120 °C, 4 h. White prisms; mp 126—127 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.47 (1H, s), 7.60—7.70 (2H, m), 7.72 (1H, s), 7.88—8.00 (4H, m), 8.64 (1H, s). IR (KBr) cm^{-1}: 1333, 1139. *Anal.* Calcd for $C_{17}H_{10}F_6N_2O_2S$: C, 48.58; H, 2.40; N, 6.66. Found: C, 48.85; H, 2.15; N, 6.93.

Preparation of *N*-(5-Nitro-4-trifluoromethyl-2-pyridinyl)ethanesulfonamide (4k) To a stirred solution of **3a** (16 mmol) in acetic acid (15 ml) was added dropwise fumic nitric acid (2.1 ml, 48 mmol) at 100 °C and the reaction was continued for an additional 2.5 h at 115 °C. After cooling the mixture was poured into an ice water (70 ml) to form solid. The solid were collected by filtration, washed with water and dried to give crude product, which was purified by silica gel column chromatography eluted with EtOAc to give **4k** as pale yellow solid. mp 201—202 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.27 (3H, t, J=6.9 Hz), 3.60 (2H, q, J=6.9 Hz), 7.38 (1H, s), 9.18 (1H, s), 11.83 (1H, bs). IR (KBr) cm^{-1}: 1543, 1342, 1145. *Anal.* Calcd for $C_8H_8F_3N_3O_4S$: C, 32.11; H, 2.69; N, 14.04. Found: C, 32.40; H, 2.72; N, 13.90.

Assay of PLA_2 Activity The ability of the synthetic compounds to inhibit $sPLA_2$ activity was determined in an assay system using a substrate of mixed micelles of phosphatidylcholine and cholate, according to the procedure reported by Volwerk *et al.*[20]

Dipalmitoyl phosphatidylcholine (13.6 μmol) and 0.5 M sodium cholate (120 μl) were suspended in 1880 μl of buffer containing 250 mM NaCl and 250 mM Tris–HCl, pH 8, and used as the substrate. The reaction mixture (total volume of 50 μl) contained (final concentrations) 0.2 μg/ml porcine pancreatic PLA_2 (Sigma-Aldrich), 2.7 mM dipalmitoyl-PC, 12 mM sodium cholate, 10 mM $CaCl_2$, 94 mM NaCl, 1 mg/ml bovine serum albumin (BSA), 94 mM Tris–HCl, pH 8, and the test sample at various concentrations. The reaction was started by addition of the substrate to a mixture of PLA_2 and a test sample, and incubation was continued at 37 °C for 30 min. The production of free fatty acids was measured by the ACS–ACO method.[19] After the incubation, 50 μl of colorization reagent A (NEFA C-Test Wako; 1.46 mM coenzyme A, 9 mM ATP, 3 mM 4-aminoantipyrine, 0.54 U/ml ACS, 5.4 U/ml ascorbate oxidase, 50 mM phosphonate, pH 7) was added to the reaction mixture and incubation was continued at 37 °C for another 10 min. Then 100 μl of colorization reagent B (NEFA C Test Wako; 5.5 U/ml ACO, 6.8 U/ml peroxidase, 1.2 mM 3-methyl-*N*-ethyl-*N*-(β-hydroxyethyl)aniline) was added and incubation was continued at 37 °C for another 10 min. The production of dye was evaluated by measuring the absorbance at 595 nm. Three replicates were used for each determination of $sPLA_2$ activity.

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