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Synthesis and biological evaluation of 4-fluoroproline and 4-fluoropyrrolidine-2-acetic acid derivatives as new GABA uptake inhibitors

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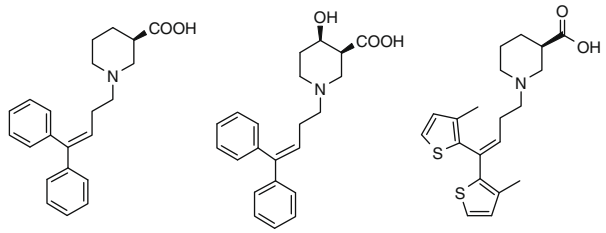
ABSTRACT

Preparation for the N-alkylated derivatives of enantiomerically pure (2*S*)-4-fluoroproline and (2*S*)-4-fluoropyrrolidine-2-acetic acid is described. The final compounds were evaluated as potential GAT-1 uptake inhibitors via cultured cell lines expressing mouse GAT-1. Compared with their corresponding 4-hydroxy compounds, these derivatives exhibited slight improvement on their inhibitory potency, but still much weaker than their corresponding compounds with no substituents at the C-4 of the pyrrolidine moiety, with the most potent affinity being about 1/15 fold as that of Tiagabine. The drastic decrease of their affinity may arise from sharp reduction of their basicity due to strong inductive effect of the 4-fluorine. However the configuration of the C-4 linking fluorine did not have much influence on their affinity for GAT-1.

1. Introduction

γ-Aminobutyric acid (GABA) plays a significant role for the inhibitory function in the mammalian central nervous system (CNS), as approximate 40% of synapses in the CNS are GABA'ergic.[1–3] A decrease of GABA'ergic neurotransmission appears to be involved in the development and outbreak of several diseases, such as epilepsy,[4] Huntington's chorea,[5] and Parkinson's disease.[6] GABA uptake protein (GAT) inhibitors have shown great promise for the treatment of related diseases, as they have less influences on GABA receptor system.[7–9]



(*R*)-SK&F-89976-A (**1**) (*RS*)-SK&F-100591 (**2**) Tiagabine (**3**)

Figure 1. Representatives of potent GABA uptake inhibitors.

The substitution of the nitrogen atoms in conformationally strained GABA analogues with appropriate bulky lipophilic groups led to the discovery of very potent GABA uptake inhibitors for GAT-1, i.g. (*R*)-SK&F 89976-A (**1**), (*RS*)-*cis*-SK&F 100591-A (**2**)[10] and Tiagabine (**3**).[11] Since then, many similar potent derivatives with different bulky lipophilic groups from those of **1** and **3** have been synthesized and intensively investigated for their pharmacology (Fig. 1).[12–15]

Wanner's group found that the novel compounds **4–7** with high (2*S*)-homoproline in place of classic (*R*)-nipecotic acid, exhibited selectively potent GAT-1 inhibition, the compound **7** being only slightly weaker than Tiagabine (Fig. 2).[16] While introduction of a hydroxyl group into the C-4 position of the pyrrolidine moiety led to a drastic decrease of its potency, for example, by comparison of **4–7** with the compounds **8–10**, respectively (Fig. 2).[17]

Due to the unique physicochemical properties of fluorine, such as highest electronegativity, strong lipophilicity, extremely low polarizability, and the ability to participate in a hydrogen bond,[18] organofluorine compounds have exerted a range of applications in medicines over the past several decades.[19–21] Thus, following my previous work, 4-hydroxy group at the C-4 position of the pyrrolidine moiety was substituted by fluorine. Meantime, two typically bulky lipophilic groups **a–b** were chosen as their N-substituents. Therefore, two different series of (2*S*)-pyrrolidine derivatives (2*S*,4*R*)-**11a–b** and (2*S*,4*S*)-**12a–b** aimed at GAT-1, were prepared in enantiomerically pure form and evaluated as potential GAT-1 inhibitors (Fig. 3).

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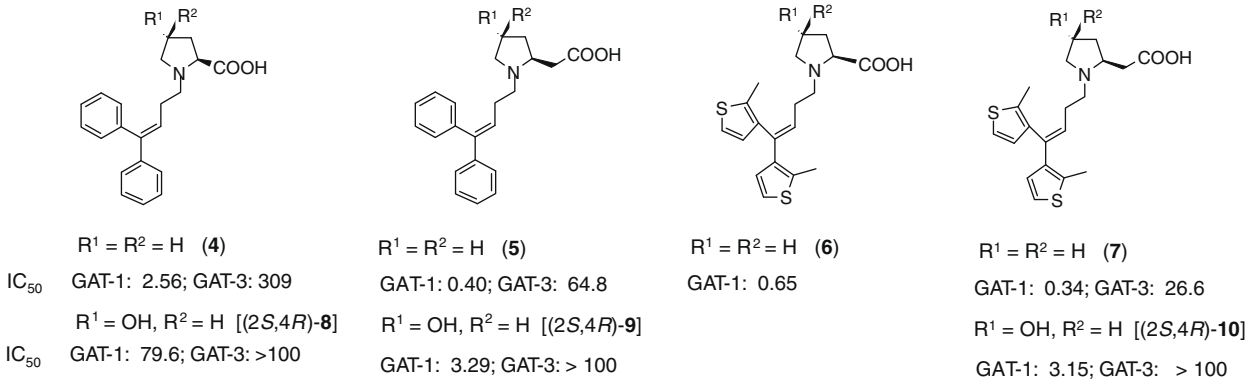


Figure 2. GABA uptake inhibitors with a pyrrolidine moiety (IC_{50} concentration: μM).

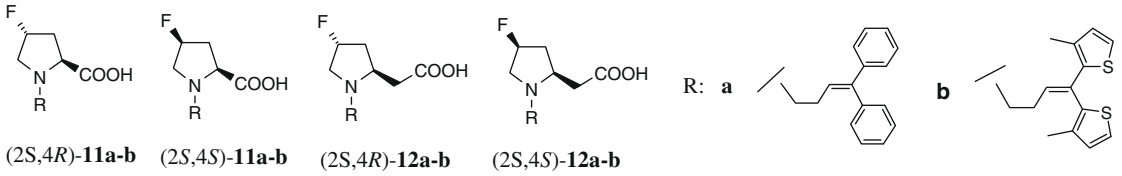


Figure 3. New target compounds.

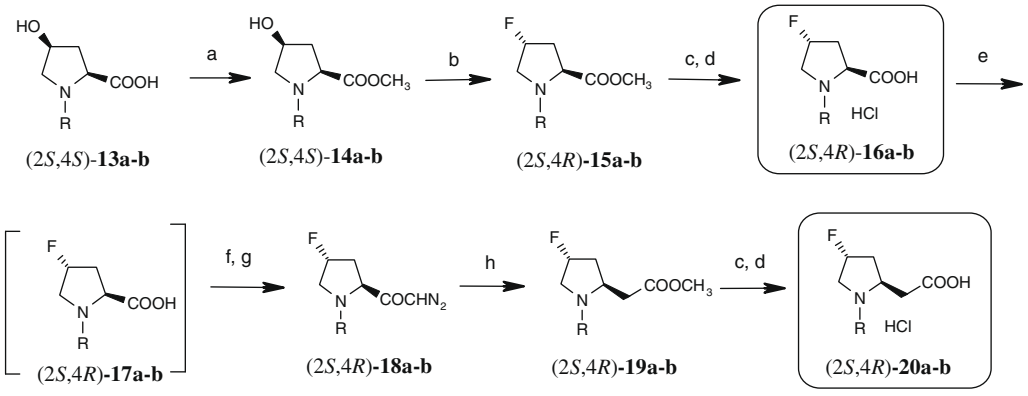
2. Chemistry

The acids (2*S*,4*S*)-**13a–b** were treated with CH_2N_2[22] in CH_2Cl_2 at 0 °C readily to give their corresponding esters in excellent yields (Scheme 1). The conversion of the alcohol (2*S*,4*S*)-**14a–b** into their corresponding fluorides with the stereo inversion at C-4 center was accomplished in good yields by perfluoro-1-butanesulfonyl fluoride and tetrabutylammonium triphenyldifluorosilicate (PBSF-TBAT) in toluene at the presence of *i*Pr_2EtN according to the procedure in our recent publication.[23] The esters (2*S*,4*R*)-**15a–b** were subject to sodium hydroxyl in ethanol, and salted by dried HCl methanol solution in CH_2Cl_2 to give target compounds (2*S*,4*R*)-**16a–b**.

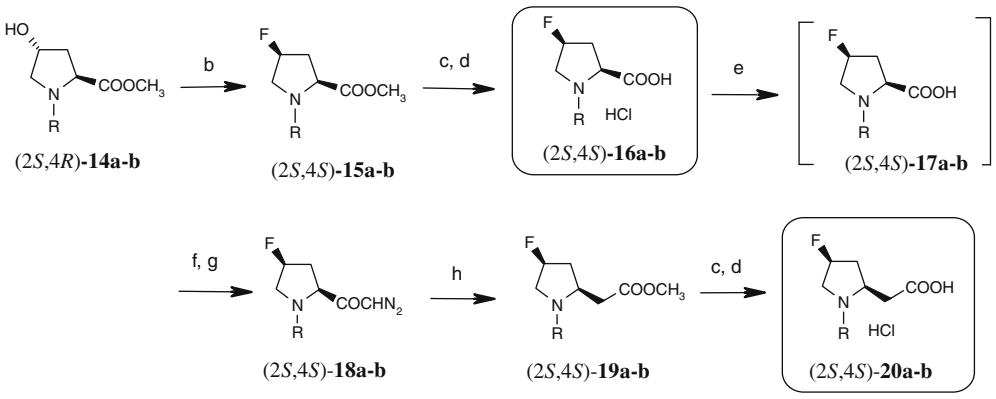
(2*S*,4*R*)-**16a–b** were titrated with anhydrous 1 N NaOH ethanol solution to offer the free acids (2*S*,4*R*)-**17a–b**. By employing $(COCl)_2$ (or $SOCl_2$) and then CH_2N_2, however transformation of the free acids (2*S*,4*R*)-**17a–b** into their diazoketones (2*S*,4*R*)-**18a–b** failed. We speculated that the tertiary amine in the adjacent position of the acid group may affect the formation of the desired acid chloride.[24] Penke et al.[25] reported that by applying a mixed carboxylic-carbonic anhydride, the chain extension of the carboxylic acid can be achieved by Arndt–Eistert reaction. Thus, (2*S*,4*R*)-**17a–b** was reacted with *iso*butyl chloroformate at −20 °C in the presence of TEA, and further with CH_2N_2 to afforded (2*S*,4*R*)-**18a–b**. Then wolff's rearrangement of the diazo compounds in methanol catalyzed by $AgNO_3$–TEA led to the desired β-amino acid esters (2*S*,4*R*)-**19a–b**. Finally (2*S*,4*R*)-**20a–b** were obtained by the same procedure to synthesize (2*S*,4*R*)-**16a–b**.

Starting from (2*S*,4*R*)-**14a–b** (Scheme 2), the compounds (2*S*,4*S*)-**16a–b** as well as (2*S*,4*S*)-**20a–b** could be obtained via the same synthetic methods as described in Scheme 1.

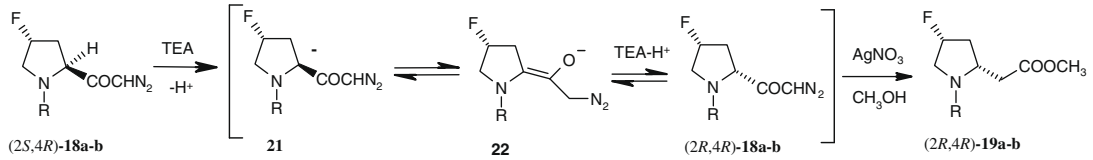
It is know that Wolff's rearrangement of diazoketones proceeds occasionally with partial inversion of the chiral center next to the carbonyl group, which depends on its applied catalysts, solvents or structural characteristics.[26–30] In our case no epimized products were observed for the rearrangement of (2*S*,4*S*)-**18a–b** induced by $AgNO_3$ (0.2 equiv)–TEA (0.1 equiv) in methanol, whereas in the same condition the rearrangement of (2*S*,4*R*)-**18a** as well as (2*S*,4*R*)-**18b** gave (2*S*,4*R*)-**19a** (64%) and epimized (2*R*,4*R*)-**19a** (12%), (2*S*,4*R*)-**19b** (54%) and epimized (2*R*,4*R*)-**19b** (9%), respectively. We suspected that owing to the strong induction of the 4-fluorine, the α-proton of the diazoketone became more active so



Scheme 1. Synthesis for (2*S*,4*R*)-**16a–b** and (2*S*,4*R*)-**20a–b**: (a) CH_2N_2, 0 °C; (b) PBSF-TBAT, *i*Pr_2EtN; (c) 4 N NaOH, C_2H_5OH, then 4 N HCl pH 5–6; (d) 37% HCl; (e) 1 N NaOH, C_2H_5OH; (f) *i*butyl chloroformate, TEA, −20 °C; (g) CH_2N_2, 0 °C; (h) $AgNO_3$–TEA, CH_3OH.



Scheme 2. Synthesis for (2*S*,4*S*)-**16a–b** and (2*S*,4*S*)-**20a–b**: reagents and conditions for (a)–(h) were identical to those in Scheme 1.



Scheme 3. Assumed epimized procedure during the Wolff's rearrangement of (2*S*,4*R*)-**18a–b**.

that TEA as a base could deprive the proton to form carbonion **21**, whose tautomer **22** further afforded the epimer (2*R*,4*R*)-**18a–b** (Scheme 3). To verify our assumption, a large amount of TEA (2.0 equiv) was used in the rearrangement of (2*S*,4*R*)-**19a–b**, interestingly, epimized (2*R*,4*R*)-**19a** (43%) and (2*R*,4*R*)-**19b** (45%) respectively as major were obtained, but (2*S*,4*R*)-**19a** (35%) and (2*S*,4*R*)-**19b** (29%), respectively as minor. From these results, we may postulate that (2*R*,4*R*)-**18a–b** with the favorite *cis* conformation tended to become dominant at the presence of excessive TEA (2.0 equiv), leading to the epimized products as major, in contrast (2*S*,4*S*)-**19a–b** with more stable *cis* conformation did not tend to epimize to *trans* at the presence of a small amount of TEA (0.1 equiv).

3. Results and discussion

The compounds (2*S*,4*R*)-**16a–b**, (2*S*,4*S*)-**16a–b**, (2*S*,4*R*)-**20a–b** and (2*S*,4*S*)-**20a–b** were investigated as potential inhibitors of GABA transport proteins on culture cell lines expressing mouse GAT-1 in transport bioassay[31] with (*R*)-Tiagabine (**3**) as a control. The IC_{50} values of GABA uptake in vitro are presented in Table 1.

Compared with the compound (2*S*,4*R*)-**8** (IC_{50} = 79.6 μM), (2*S*,4*R*)-**16a** with fluorine in place of hydroxyl group exhibited a slight improvement on its potency (IC_{50} = 67.4 μM, IC_{50} of Tiagabine = 0.191 μM as a control). But in contrast with the compound **6** (IC_{50} = 0.645 μM;[16] IC_{50} of Tiagabine = 0.159 μM as a control), both new derivatives (2*S*,4*R*)-**16b** (IC_{50} = 12.8 μM) and (2*S*,4*S*)-**16b** (IC_{50} = 18.0 μM), whatever its configuration of the C-4 is *R* or *S*, exhibited sharp decrease in their inhibitory potency on GAT-1. By comparison with the compound (2*S*,4*R*)-**9** [IC_{50} of (2*S*,4*R*)-**9** = 3.29 μM; IC_{50} of (2*S*,4*S*)-**9** = 4.92 μM], their 4-fluoro derivatives presented approximately equivalent affinity [IC_{50} of (2*S*,4*R*)-**20a** = 3.96 μM; IC_{50} of (2*S*,4*S*)-**20a** = 5.81 μM]. Furthermore, as referred to the compound (2*S*,4*R*)-**10** [IC_{50} of (2*S*,4*R*)-**10** = 3.15 μM; IC_{50} of (2*S*,4*S*)-**10** = 5.14 μM], this replacement of a hydroxyl group with a fluorine at C-4 position only caused slightly improved affinity [IC_{50} of (2*S*,4*R*)-**20b** = 2.97 μM; IC_{50} of (2*S*,4*S*)-**20b** = 4.94 μM]. Further contrary to the compounds **5** (IC_{50} = 0.40 μM) and **7** (IC_{50} = 0.343 μM), the tested **20a–b** series of the 4-fluoro analogues still displayed much weaker potency (IC_{50} ranged from 2.97 to 5.81 μM). Moreover, it can be seen from Table 1 that the configuration at C-4 linking fluorine appears to have no much influences for their affinity.

Because of the strong inductive effect, β-substituted fluorine of an amino group tends to dramatically reduce its basicity.[32,33] The pK_b data revealed that the basicity of β-trifluoroethylamine sharply decreased nearly at five orders of magnitude in comparison with that of ethylamine. From a series of statistical data,[32,33] we may infer that, in our case, this single fluorine β-substitution lead to a drop of the basicity at not less than one order of magnitude. It is known now that an amine functional group, preferably a secondary amine, is an essentially pharmacophoric requirement for the affinity of inhibitors to GABA transport proteins.[34] Thus, we may assume that the harsh reduction of their bascity accounts for the big loss of their affinity to GAT-1.

Table 1
GABA uptake IC_{50} of compounds **16a–b** and **20a–b**

No.	Compound no.	IC_{50} (μM)
1	**3** (Tiagabine)	0.191
2	(2*S*,4*R*)-**16a**	67.4
3	(2*S*,4*S*)-**16a**	>100
4	(2*S*,4*R*)-**16b**	12.8
5	(2*S*,4*S*)-**16b**	18.0
6	(2*S*,4*R*)-**20a**	3.96
7	(2*S*,4*S*)-**20a**	5.81
8	(2*S*,4*R*)-**20b**	2.97
9	(2*S*,4*S*)-**20b**	4.94

4. Conclusion

In conclusion, the introduction of fluorine into the C-4 position of the (2*S*)-proline derivatives and the (2*S*)-pyrrolidine-2-acetic acid derivatives drastically decreased their inhibitory potency at GAT-1, but exhibited slight improvement on their potency compared with those corresponding 4-hydroxy derivatives. We assumed that strong induction of the fluorine at β-position of the amine functional group may cause drastic drop of their basicity and decrease their affinity to GAT-1. Our results manifested that weak basicity of the amino functional group is detrimental to their

potent inhibition but the configuration of the carbon-4 linking fluorine did not have large impact.

5. Experimental part

5.1. General mark

Reagents were purchased from commercial sources and used without further purification unless otherwise indicated. Toluene and diisopropylethylamine (*i*Pr_2NEt) were distilled from sodium under nitrogen; Dichloromethane was distilled from CaH_2 under nitrogen, while triethylamine (TEA) was refluxed with benzoyl chloride and distilled under nitrogen before use. TBAT was prepared from triphenylsilanol according the literature method.[35] Melting points were determined with a Büchi 540 Melting Point apparatus and are uncorrected. If nothing else is stated, all packing for flash chromatography was silica gel (mesh 300–400). Optical rotation was measured at λ 589 cm^{-1} of Na light. IR spectra were run on FI-IR Spectrometer (Perkin Elmer) with oily samples as film and with solid samples as pellets. ^{1}H NMR spectra were recorded on Unity-600 if nothing else is specified, and MS spectra on Agilent 1100 Series.

5.2. General procedure 1 for the preparation of (2*S*,4*S*)-methyl 1-(4,4-diphenyl-3-butenyl)-4-hydroxylpyrrolidine -2-carboxylate [(2*S*,4*S*)-14a] and (2*S*,4*S*)-methyl 1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-4-hydroxylpyrrolidine -2-carboxylate [(2*S*,4*S*)-14b]

To a solution of the respective **13a–b** (1 equiv) in CH_2Cl_2 (5 ml per mmol), freshly prepared CH_2N_2 (3 equiv) in CH_2Cl_2 (4 ml per mmol) was added at 0 °C. The excessive CH_2N_2 was decomposed by slow addition of acetic acid. The reaction mixture was evaporated to leave an oil, which was purified by flash chromatography.

(2*S*,4*S*)-**14a**: yellow oil; yield: 93%; $[\alpha]_D^{20} = -45$ (c = 1.1, ethyl acetate) IR: $\tilde{\nu}$ 3307, 2982, 1728, 1233, 1110, 764 cm^{-1}; MS (*m*/*z*) ESI$^+$: 352 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 1.93 (dd, 1H, J = 14.1, 2.4 Hz, NCHCH_2), 2.28–2.39 (m, 3H, 1H of NCHCH_2 and 2H of NCH_2CH_2), 2.57 (dd, 1H, J = 10.0, 4.2 Hz, NCH_2CHOH), 2.60–2.66 (m, 1H, NCH_2CH_2), 2.82 (dt, 1H, J = 12.1, 7.9 Hz, NCH_2CH_2), 3.01 (d, 1H, J = 9.9 Hz, NCH_2CHOH), 3.26 (dd, 1H, J = 9.3, 3.7 Hz, NCH), 3.71 (s, 3H, OCH_3), 4.26 (t, 1H, J = 4.4 Hz, CHOH), 6.09 (t, 1H, J = 7.2 Hz, =CHCH$_2$), 7.16–7.41 (m, 10H, $H_{aromat.}$); Calcd for $C_{22}H_{25}NO_3$ (351.44): C, 75.19; H, 7.17; N, 3.99. Found: C, 74.97; H, 7.29; N, 3.75.

(2*S*,4*S*)-**14b**: yellow oil; yield: 95%; $[\alpha]_D^{20} = -51$ (c = 0.95, ethyl acetate); IR: $\tilde{\nu}$ 3258, 3035, 2980, 1739, 1520, 1259, 1110 cm^{-1}; MS (*m*/*z*) ESI$^+$: 392 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 2.00 (s, 3H, thienyl–CH_3), 2.04 (s, 3H, thienyl–CH_3), 2.29–2.39 (m, 3H, 1H of NCHCH_2 and 2H of NCH_2CH_2), 2.56 (dd, 1H, J = 10.0, 4.6 Hz, NCH_2CHOH), 2.61 (dt, 1H, J = 12.4, 7.3 Hz, NCH_2CH_2), 2.82 (dt, 1H, J = 12.4, 8.2 Hz, NCH_2CH_2), 3.03 (d, 1H, J = 10.0 Hz, NCH_2CHOH), 3.26 (dd, 1H, J = 10.0, 3.9 Hz, NCH), 3.72 (s, 3H, OCH_3), 4.26 (t, 1H, J = 4.7 Hz, CHOH), 6.05 (t, 1H, J = 7.3 Hz, =CHCH$_2$), 6.76 (d, 1H, J = 5.5 Hz, SC=CH), 6.85 (d, 1H, J = 5.5 Hz, SC=CH), 7.05 (d, 1H, J = 5.5 Hz, SCH), 7.22 (d, 1H, J = 5.5 Hz, SCH); Calcd for $C_{20}H_{25}NO_3S_2$ (391.54): C, 61.35; H, 6.44; N, 3.58; S, 16.38. Found: C, 61.04; H, 6.18; N, 3.25; S, 16.59.

5.3. General procedure 2 for the preparation of methyl 1-(4,4-diphenyl-3-butenyl)-4-fluoropyrrolidine-2-carboxylate (15a) and methyl 1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-4-fluoropyrrolidine-2-carboxylate (15b)

To a mixture of the respective alcohols **14a–b** (1 equiv) and TBAT (0.8 equiv), *i*Pr_2EtN (2.5 equiv) and toluene (8 ml per mmol of alcohol) were added in turns at rt. Then PBSF (2.2 equiv) was introduced into the resulting mixture. The stirring was continued until TLC revealed complete conversion. The reaction mixture was concentrated under reduced pressure. The residual was mixed with a little bit of silica gel, dried in ventilation, and finally purified by flash chromatography to give the corresponding fluoride.

(2*S*,4*R*)-**15a**: slightly yellow oil; yield: 82%; $[\alpha]_D^{18} = -43$ (c = 0.88, ethyl acetate); IR: $\tilde{\nu}$ 3054, 2952, 1734, 1443, 1200, 761, 701 cm^{-1}; MS (*m*/*z*) ESI$^+$: 354 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 2.11–2.23 (m, 1H, NCHCH_2), 2.28–2.38 (m, 3H, 2H of NCH_2CH_2 and 1H of NCHCH_2), 2.63 (dt, 1H, J = 11.9, 7.5 Hz, NCH_2CH$_2$), 2.71 (dd, 1H, J = 30.8, 12.4 Hz, NCH_2CHF), 2.84 (dt, 1H, J = 11.9, 7.5 Hz, NCH_2CH$_2$), 3.41 (ddd, 1H, J = 30.2, 11.5, 5.0 Hz, NCH_2CHF), 3.57 (t, 1H, J = 7.3 Hz, NCHCO), 3.69 (s, 3H, OCH_3), 5.18 (d, 1H, J = 55.4 Hz, CHF), 6.08 (t, 1H, J = 7.3 Hz, =CHCH$_2$), 7.15–7.39 (m, 10H, $H_{aromat.}$); Calcd $C_{22}H_{24}FNO_2$ (353.24): C, 74.76; H, 6.85; N, 3.96. Found: C, 74.48; H, 7.22; N, 3.81.

(2*S*,4*S*)-**15a**: slightly yellow oil; yield: 85%; $[\alpha]_D^{21} = -29$ (c = 0.92, ethyl acetate); IR: $\tilde{\nu}$ 3104, 3058, 2930, 2854, 1739, 1436, 1158, 711 cm^{-1}; MS (*m*/*z*) ESI$^+$: 354 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 2.20–2.36 (m, 3H, 2H of NCH_2CH_2 and 1H of NCHCH$_2$), 2.43–2.56 (m, 3H, NCHCH_2, NCH_2CH$_2$ and NCH_2CHF), 2.92 (dt, 1H, J = 12.4, 7.3 Hz, NCH_2CH$_2$), 3.19 (t, 1H, J = 7.4 Hz, NCHCO), 3.35 (dd, 1H, J = 21.0, 11.5 Hz, NCH_2CHF), 3.71 (s, 3H, OCH_3), 5.10 (dt, 1H, J = 54.5, 5.0 Hz, CHF), 6.07 (t, 1H, J = 7.3 Hz, =CHCH$_2$), 7.15–7.38 (m, 10H, $H_{aromat.}$); Calcd for $C_{22}H_{24}FNO_2$ (353.44): C, 74.76; H, 6.85; N, 3.96. Found: C, 74.69; H, 7.28; N, 3.73.

(2*S*,4*R*)-**15b**: yellow oil; yield: 78%; $[\alpha]_D^{18} = -47$ (c = 1.2, ethyl acetate); IR: $\tilde{\nu}$ 3105, 3060, 2952, 1738, 1435, 1199, 713 cm^{-1}; MS (*m*/*z*) ESI$^+$: 394 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 2.01 (s, 3H, thienyl–CH_3), 2.04 (s, 3H, thienyl–CH_3), 2.11–2.24 (m, 1H, NCHCH_2), 2.29–2.39 (m, 3H, 2H of NCH_2CH_2 and 1H of NCHCH_2), 2.60–2.66 (m, 1H, NCH_2CH$_2$), 2.73 (dd, 1H, J = 30.9, 11.4 Hz, NCH_2CHF), 2.82–2.89 (m, 1H, NCH_2CH$_2$), 3.43 (ddd, 1H, J = 30.6, 11.6, 4.5, NCH_2CHF), 3.58 (t, 1H, J = 7.2 Hz, NCHCO), 3.71 (s, 3H, OCH_3), 5.20 (br d, 1H, J = 55.3 Hz, CHF), 6.05 (t, 1H, J = 7.1 Hz, =CHCH$_2$), 6.75 (d, 1H, J = 4.9 Hz, SC=CH), 6.84 (d, 1H, J = 4.9 Hz, SC=CH), 7.05 (d, 1H, J = 4.9 Hz, SCH), 7.21 (d, 1H, J = 4.9 Hz, SCH); Calcd for $C_{20}H_{24}FNO_2S_2$ (393.53): C, 61.04; H, 6.15; N, 3.56; S, 16.30. Found: C, 60.84; H, 6.34; N, 3.37; S, 15.93.

(2*S*,4*S*)-**15b**: yellow oil; yield: 80%; ^{1}H NMR and IR spectra were identical with those in the literature.[23]

5.4. General procedure 3 for the preparation of 2-diazoacetyl-1-(4,4-diphenyl-3-butenyl)-4-fluoropyrrolidine (18a) and 1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-2-diazoacetyl-4-fluoropyrrolidine (18b)

The respective salt of **16a–b** (1 equiv) was titrated with 1 N NaOH (1 equiv) in anhydrous ethanol, and the free acid **17a–b** was obtained after removal of ethanol. The acid was then dissolved in CH_2Cl_2 solution (5 ml per mmol), TEA (1.2 equiv) was added and then *iso*butanyl chloroformate (1.1 equiv) was introduced at −20 °C. After stirring was continued for 1.5 h, freshly prepared CH_2N_2 (5 equiv) in CH_2Cl_2 was added at 0 °C. Two hours later, the reaction mixture was evaporated under reduced pressure at rt in a hood with good ventilation. Purification by flash chromatography gave each diazo compound.

(2*S*,4*R*)-**18a**: yellow oil; yield: 81%; $[\alpha]_D^{18} = -53$ (c = 0.90, ethyl acetate); MS (*m*/*z*) ESI$^+$ 364 [M+1]$^+$, 386 [M+Na]$^+$; IR: $\tilde{\nu}$ 3101, 3023, 2964, 2104, 1639, 1343, 762, 701 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.80–1.93 (m, 1H, NCHCH_2), 2.29 (q, 2H, J = 7.2 Hz, NCH$_2$CH_2), 2.38 (ddd, 1H, J = 20.6, 14.7, 6.9 Hz, NCHCH_2), 2.56–2.66 (m, 2H, NCH_2CHF and NCH_2CH$_2$), 2.78 (dt, 1H, J = 11.6, 7.3 Hz, NCH_2CH$_2$), 3.34 (ddd, 1H, J = 32.0, 12.4, 4.6 Hz, NCH_2CHF), 3.43 (dd, 1H,

J = 9.8, 7.3 Hz, NC*H*CO), 5.08 (d, 1H, J = 55.4 Hz, C*H*F), 5.82 (s, 1H, C*H*N_2), 6.11 (t, 1H, J = 7.3 Hz, =C*H*CH_2), 7.16–7.41 (m, 10H, $H_{aromat.}$); Calcd for $C_{22}H_{22}FN_3O$ (363.44): C, 72.71; H, 6.10; N, 11.56. Found: C, 72.56; H, 6.26; N, 11.34.

(2*S*,4*S*)-**18a**: yellow oil; yield: 56%; $[\alpha]_D^{21} = -56$ (c = 1.1, ethyl acetate); MS (m/z) ESI$^+$: 364 [M+1]$^+$; IR: $\tilde{\nu}$ 3116, 3055, 2969, 2808, 2103, 1635, 1348, 763, 702 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.08 (ddd, 1H, J = 27.0, 15.6, 5.0 Hz, NCHC*H*$_2$), 2.28–2.39 (m, 3H, 2H of NCH$_2$C*H*$_2$ and 1H of NCHC*H*$_2$), 2.43–2.59 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$CHF), 2.79 (dt, 1H, J = 12.0, 8.0 Hz, NC*H*$_2$CH$_2$), 3.11 (dd, 1H, J = 11.0, 5.0 Hz, NC*H*CO), 3.32 (dd, 1H, J = 17.5, 12.0 Hz, NC*H*$_2$CHF), 5.08 (dt, 1H, J = 53.1, 3.8 Hz, C*H*F), 6.05 (s, 1H, C*H*N_2), 6.11 (t, 1H, J = 7.2 Hz, =C*H*CH_2), 7.14–7.44 (m, 10H, $H_{aromat.}$); Calcd for $C_{22}H_{22}FN_3O$ (363.44): C, 72.71; H, 6.10; N, 11.56. Found: C, 72.38; H, 6.36; N, 11.29.

(2*S*,4*R*)-**18b**: yellow oil; yield: 67%; $[\alpha]_D^{24} = -62$ (c = 0.89, ethyl acetate); MS (m/z) ESI$^+$: 402 [M−1]$^+$; IR: $\tilde{\nu}$ 3106, 2823, 2828, 2105, 1635, 1346, 714 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.81–1.95 (m, 4H, 1H of NCHC*H*$_2$ and 3H of thienyl–C*H*$_3$), 2.05 (s, 3H, thienyl–C*H*$_3$), 2.28–2.44 (m, 3H, 2H of NCH$_2$C*H*$_2$ and 1H of NCHC*H*$_2$), 2.55–2.66 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$CHF), 2.78–2.84 (m, 1H, NC*H*$_2$CH$_2$), 3.36 (ddd, 1H, J = 32.1, 12.5, 4.4 Hz, NC*H*$_2$CHF), 3.44 (dd, 1H, J = 9.2, 7.8 Hz, NC*H*CO), 5.10 (d, 1H, J = 54.5 Hz, C*H*F), 5.93 (s, 1H, C*H*N_2), 6.10 (t, 1H, J = 7.3 Hz, =C*H*CH_2), 6.76 (d, 1H, J = 5.0 Hz, SC*H*), 6.86 (d, 1H, J = 5.0 Hz, SC*H*), 7.06 (d, 1H, J = 5.2 Hz, SC=C*H*), 7.24 (d, 1H, J = 5.2 Hz, SC=C*H*); Calcd for $C_{20}H_{22}FN_3OS_2$ (403.53): C, 59.53; H, 5.50; N, 10.41; S, 15.89. Found: C, 59.41; H, 5.64; N, 10.18; S, 15.60.

(2*S*,4*S*)-**18b**: yellow oil: yield: 62%; $[\alpha]_D^{18} = -75$ (c = 0.80, ethyl acetate); MS (m/z) ESI$^+$: 404 [M+1]$^+$; IR: $\tilde{\nu}$ 3111, 2922, 2811, 2103, 1688, 1635, 1348, 712 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 3H, thienyl–C*H*$_3$), 2.04 (s, 3H, thienyl–C*H*$_3$), 2.05–2.15 (m, 1H, NCHC*H*$_2$), 2.32–2.42 (m, 3H, 2H of NCH$_2$C*H*$_2$ and 1H of NCHC*H*$_2$), 2.44–2.55 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$CHF), 2.82 (dt, 1H, J = 12.0, 8.0 Hz, NC*H*$_2$CH$_2$), 3.11 (dd, 1H, J = 10.4, 5.5 Hz, NC*H*CO), 3.34 (ddd, 1H, J = 18.3, 11.4, 1.5 Hz, NC*H*$_2$CHF), 5.09 (dt, 1H, J = 53.5, 4.0 Hz, C*H*F), 6.08–6.14 (m, 2H, =C*H*CH_2 and C*H*N_2), 6.76 (d, 1H, J = 5.2 Hz, SC*H*), 6.86 (d, 1H, J = 5.2 Hz, SC*H*), 7.06 (d, 1H, J = 5.2 Hz, SC=C*H*), 7.23 (d, 1H, J = 5.2 Hz, SC=C*H*); Calcd for $C_{20}H_{22}FN_3OS_2$ (403.53): C, 59.53; H, 5.50; N, 10.41; S, 15.89. Found: C, 59.33; H, 5.83; N, 10.25; S, 15.52.

5.5. General procedure 4 for the preparation of methyl 1-(4,4-diphenyl-3-butenyl)-4-fluoropyrrolidine-2-acetate (19a) and methyl 1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-4-fluoropyrrolidine-2-acetate (19b)

Method A: To a solution of the respective **18a–b** (1 equiv) in MeOH (7 ml), a solution of $AgNO_3$ (0.2 equiv) and TEA (0.1 equiv) in MeOH (4 ml) was added at rt. The mixture turned to be brown within a few minutes, and then warmed to 60 °C for 30 min. The reaction mixture was decolorized with charcoal and concentrated. The resulting oil was purified by flash chromatography to give the desired ester.

Method B is the same as Method A but TEA (2.0 equiv) was employed.

(2*S*,4*R*)-**19a** by Method A from (2*S*,4*R*)-**18a**: yellow oil, R_f small; yield: 64%; $[\alpha]_D^{20} = -37$ (c = 0.92, ethyl acetate); IR: $\tilde{\nu}$ 3058, 3021, 2949, 2854, 1735, 1443, 1158, 761, 704 cm^{-1}; MS (m/z) ESI$^+$: 368 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 1.72–1.84 (m, 1H, NCHC*H*$_2$), 2.21–2.32 (m, 4H, 2H of NCH$_2$C*H*$_2$, 1H of NCHC*H*$_2$ and 1H of C*H*$_2$COO), 2.41–2.53 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$CHF), 2.63 (dd, 1H, J = 13.5, 3.9 Hz, C*H*$_2$COO), 2.83–2.89 (m, 1H, NC*H*$_2$CH$_2$), 3.07–3.13 (m, 1H, NC*H*CH$_2$), 3.39 (ddd, 1H, J = 28.0, 11.9, 5.0 Hz, NC*H*$_2$CHF), 3.65 (s, 3H, OC*H*$_3$), 5.08 (dt, 1H, J = 55.0, 5.0 Hz, C*H*F), 6.08 (t, 1H, J = 7.3 Hz, =C*H*CH_2), 7.15–7.40 (m, 10H, $H_{aromat.}$); Calcd for $C_{23}H_{26}FNO_2$ (367.46): C, 75.18; H, 7.13; N, 3.81. Found: C, 74.85; H, 7.42; N, 3.66.

(2*R*,4*R*)-**19a** by Method A from (2*S*,4*R*)-**18a**: yellow oil, R_f large; yield: 12%; $[\alpha]_D^{22} = +47$ (c = 0.88, ethyl acetate); IR: $\tilde{\nu}$ 3058, 3023, 2951, 2847, 1738, 1443, 1163, 763, 701 cm^{-1}; MS (m/z) ESI$^+$: 368 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 1.85 (ddd, 1H, J = 31.6, 15.0, 6.9 Hz, NCHC*H*$_2$), 2.22–2.49 (m, 6H, NCHC*H*$_2$, NCH$_2$C*H*$_2$, C*H*$_2$COO, NC*H*$_2$CHF and 2H of NCH$_2$CH$_2$), 2.70 (dd, 1H, J = 15.0, 3.9 Hz, C*H*$_2$COO), 2.78–2.91 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*CH$_2$), 3.26 (dd, 1H, J = 20.2, 11.5 Hz, NC*H*$_2$CHF), 3.66 (s, 3H, OC*H*$_3$), 5.06 (dt, 1H, J = 55.5, 4.8 Hz, C*H*F), 6.08 (t, 1H, J = 7.3 Hz, =C*H*CH_2), 7.16–7.39 (m, 10H, $H_{aromat.}$).

(2*S*,4*R*)-**19a** by Method B from (2*S*,4*R*)-**18a**; yield: 35%.

(2*R*,4*R*)-**19a** by Method B from (2*S*,4*R*)-**18a**: yield: 43%.

(2*S*,4*S*)-**19a** by Method A from (2*S*,4*S*)-**18a**: yellow oil; yield: 82%; $[\alpha]_D^{20} = -45$ (c = 1.3, ethyl acetate); IR: $\tilde{\nu}$ 3023, 2951, 2801, 1739, 1436, 762, 702 cm^{-1}; MS (m/z) ESI$^+$: 368 [M+1]$^+$; ^{1}H NMR ($CDCl_3$, 400 Mz): δ 1.84–1.96 (m, 1H, NCHC*H*$_2$), 2.27–2.62 (m, 6H, NCHC*H*$_2$, NCH$_2$C*H*$_2$, C*H*$_2$COO, NC*H*$_2$CHF and 2H of NCH$_2$CH$_2$), 2.75 (dd, 1H, J = 15.0, 3.9 Hz, C*H*$_2$COO), 2.80–2.99 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*CH$_2$), 3.28 (dd, 1H, J = 17.5, 11.6 Hz, NC*H*$_2$CHF), 3.69 (s, 3H, OC*H*$_3$), 5.08 (br d, 1H, J = 54.8 Hz, C*H*F), 6.09–6.14 (m, 1H, =C*H*CH_2), 7.16–7.43 (m, 10H, $H_{aromat.}$); Calcd for $C_{23}H_{26}FNO_2$ (367.46): C, 75.18; H, 7.13; N, 3.81. Found: C, 75.43; H, 6.92; N, 3.59.

(2*S*,4*R*)-**19b** by Method A from (2*S*,4*R*)-**18b**: yellow oil, R_f small; yield: 54%; $[\alpha]_D^{32} = -40$ (c = 0.58, ethyl acetate); IR: $\tilde{\nu}$ 3101, 2924, 2855, 1743, 1456, 710 cm^{-1}; MS (m/z) ESI$^+$: 408 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 1.70–1.82 (m, 1H, NCHC*H*$_2$), 2.00 (s, 3H, thienyl–C*H*$_3$), 2.03 (s, 3H, thienyl–C*H*$_3$), 2.23–2.34 (m, 4H, 2H of NCH$_2$C*H*$_2$, 1H of NCHC*H*$_2$ and 1H of C*H*$_2$COO), 2.42–2.56 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$CHF), 2.65 (dd, 1H, J = 14.8, 3.9 Hz, C*H*$_2$COO), 2.86 (dt, 1H, J = 11.9, 8.2 Hz, NC*H*$_2$CH$_2$), 3.08–3.14 (m, 1H, NC*H*CH$_2$), 3.41 (ddd, 1H, J = 28.0, 11.6, 5.2 Hz, NC*H*$_2$CHF), 3.65 (s, 3H, OC*H*$_3$), 5.09 (br d, 1H, J = 55.4 Hz, C*H*F), 6.04 (d, 1H, J = 7.3 Hz, =C*H*CH_2), 6.76 (d, 1H, J = 5.0 Hz, SC*H*), 6.84(d, 1H, J = 5.0 Hz, SC*H*), 7.05 (d, 1H, J = 5.1 Hz, SC=C*H*), 7.21 (d, 1H, J = 5.1 Hz, SC=C*H*); Calcd for $C_{21}H_{26}FNO_2S_2$ (407.56): C, 61.89; H, 6.43; N, 3.45; S, 15.73. Found: 61.81; H, 6.21; N, 3.28; S, 15.46.

(2*R*,4*R*)-**19b** by Method A from (2*S*,4*R*)-**18b**: yellow oil, R_f large; yield: 9%; $[\alpha]_D^{32} = +75$ (c = 0.96, ethyl acetate); IR: $\tilde{\nu}$ 3101, 2925, 2855, 1733, 1436, 712 cm^{-1}; MS (m/z) ESI$^+$: 408 [M+1]$^+$; ^{1}H NMR ($CDCl_3$): δ 1.84 (ddd, 1H, J = 31.2, 15.1, 6.6 Hz, NCHC*H*$_2$), 2.03 (s, 3H, thienyl–C*H*$_3$), 2.04 (s, 3H, thienyl–C*H*$_3$), 2.26–2.50 (m, 6H, NCHC*H*$_2$, NCH$_2$C*H*$_2$, C*H*$_2$COO, NC*H*$_2$CHF and 2H of NCH$_2$CH$_2$), 2.72 (dd, 1H, J = 15.2, 4.1 Hz, C*H*$_2$COO), 2.78–2.92 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*CH$_2$), 3.29 (dd, 1H, J = 20.3, 11.5 Hz, NC*H*$_2$CHF), 3.67 (s, OC*H*$_3$), 5.08 (dt, 1H, J = 54.5, 4.6 Hz, C*H*F), 6.05 (t, 1H, J = 7.3 Hz, =C*H*CH_2), 6.76 (d, 1H, J = 5.5 Hz, SC*H*), 6.84 (d, 1H, J = 5.0 Hz, SC*H*), 7.05 (d, 1H, J = 5.5 Hz, SC=C*H*), (d, 1H, J = 5.0 Hz, SC=C*H*); Calcd for $C_{21}H_{26}FNO_2S_2$ (407.56): C, 61.89; H, 6.43; N, 3.45; S, 15.73. Found: C, 61.95; H, 6.24; N, 3.17; S, 15.42.

(2*S*,4*R*)-**19b** by Method B from (2*S*,4*R*)-**18b**: yield: 29%.

(2*R*,4*R*)-**19b** by Method B from (2*S*,4*R*)-**18b**: yield: 45%.

(2*S*,4*S*)-**19b** by Method A from (2*S*,4*S*)-**18b**: yellow oil; yield: 75%; $[\alpha]_D^{20} = -72$ (c = 1.1, ethyl acetate); IR: $\tilde{\nu}$ 3087, 3063, 2952, 2836, 1727, 739, 700 cm^{-1}; MS (m/z) ESI$^+$: 408 [M+1]$^+$; ^{1}H NMR ($CDCl_3$, 400Mz): δ 1.80–1.96 (m, 1H, NCHC*H*$_2$), 1.98 (s, 3H, thienyl–C*H*$_3$), 2.06 (s, 3H, thienyl–C*H*$_3$), 2.27–2.65 (m, 6H, NCHC*H*$_2$, NCH$_2$C*H*$_2$, C*H*$_2$COO, NC*H*$_2$CHF and 2H of NCH$_2$CH$_2$), 2.67–2.98 (m, 3H, C*H*$_2$COO, NC*H*$_2$CH$_2$ and NC*H*CH$_2$), 3.30 (dd, 1H, J = 17.5, 11.0 Hz, NC*H*$_2$CHF), 3.70 (s, 3H, OC*H*$_3$), 5.03–5.18 (m, 1H, C*H*F), 6.04–6.09 (m, 1H, =C*H*CH_2), 6.78 (d, 1H, J = 5.1 Hz, SC*H*), 6.86 (d, 1H, J = 5.1 Hz, SC*H*), 7.07(d, 1H,

J = 5.1 Hz, SC=C*H*), 7.23 (d, 1H, J = 5.1 Hz, SC=C*H*); Calcd for $C_{21}H_{26}FNO_2S_2$ (407.56): C, 61.89; H, 6.43; N, 3.45; S, 15.73. Found: 61.72; H, 6.34; N, 3.68; S, 15.57.

5.6. General procedure 5 for the preparation of 1-(4,4-diphenyl-3-butenyl)-4-fluoropyrrolidine-2-carboxylic acid hydrochloride (16a) and 1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-4-fluoropyrrolidine-2-carboxylic acid hydrochloride (16b); 1-(4,4-diphenyl-3-butenyl)-4-fluoropyrrolidine-2-acetic acid hydrochloride (20a) and 1-[4,4-bis(3-methyl -2-thienyl)-3-butenyl]-4-fluoropyrrolidine-2-acetic acid hydrochloride (20b)

The respective ester of **15a–b** or **19a–b** (1 eq mmol) in ethanol (10 ml/mmol) was subject to 4.0 M NaOH (3 equiv) at rt for 24 h. Then the solution was acidified to pH 5–6 with aq 1.0 M HCl. After ethanol had been removed in vacuo, the residue was extracted with CH_2Cl_2, dried over Na_2SO_4 and then filtrated. After 1.0 M dried HCl CH_3OH solution (1 equiv) was added into the filter, solvents were removed under reduced pressure to afford the desired product, which further dried in vacuo.

(2*S*,4*R*)-**16a**: gray crystals; yield: 76%; mp 125–129 °C; $[\alpha]_D^{21} = -15$ (c = 1.1, ethanol); IR: $\tilde{\nu}$ 3027, 2939, 1727, 1446, 1360, 1213, 768, 703 cm^{-1}; MS (*m/z*) ESI$^+$: 340 [M−Cl]$^+$; ^{1}H NMR (CD_3OD): δ 2.19–2.32 (m, 1H, NCHC*H*$_2$), 2.44–2.53 (m, 2H, NCH$_2$C*H*$_2$), 2.68–2.77 (m, 1H, NCHC*H*$_2$), 3.25–3.34 (m, 1H, NC*H*$_2$CH$_2$), 3.44–3.55 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$CHF), 3.83 (ddd, 1H, J = 34.2, 14.1, 4.4, Hz, NC*H*$_2$CHF), 4.55 (dd, 1H, J = 12.2, 7.1 Hz, NC*H*CO), 5.36 (d, 1H, J = 53.0 Hz, C*H*F), 6.00 (t, 1H, J = 7.3 Hz, =C*H*CH$_2$), 7.08–7.20 (m, 6H, H$_{aromat.}$), 7.26–7.38 (m, 4H, H$_{aromat}$); $C_{21}H_{23}ClFNO_2$ (375.89) 1.1CH_3OH: C, 64.21; H, 6.71; N, 3.40. Found: C, 63.90; H, 6.95; N, 3.12.

(2*S*,4*S*)-**16a**: yellow crystals; yield: 85%; mp: 160–165 °C; $[\alpha]_D^{18} = -11$ (c = 0.94, ethanol); IR: $\tilde{\nu}$ 3050, 2979, 2604, 2497, 1705, 1442, 1208, 699 cm^{-1}; MS (*m/z*) ESI: 340 [M−Cl]$^+$; ^{1}H NMR (CD_3OD): δ 2.43–2.58 (m, 3H, 2H of NCH$_2$C*H*$_2$ and 1H of NCHC*H*$_2$), 2.78–2.91 (m, 1H, NCHC*H*$_2$), 3.29–3.49 (m, 3H, 2H of NC*H*$_2$CH$_2$ and 1H of NC*H*$_2$CHF), 3.86 (t, 1H, J = 15.2 Hz, NC*H*$_2$CHF), 4.41 (dd, 1H, J = 11.0, 3.7 Hz, NC*H*CO), 5.32 (d, 1H, J = 52.0 Hz, C*H*F), 6.01 (t, 1H, J = 7.3 Hz, =C*H*CH$_2$), 7.10–7.39 (m, 10H, H$_{aromat.}$); $C_{21}H_{23}ClFNO_2$ (375.89) 1.2C_2H_5OH: C, 65.17; H, 7.06; N, 3.25. Found: C, 65.01; H, 7.33; N, 2.98.

(2*S*,4*R*)-**16b**: Gray crystals; yield: 92%; mp: 106–110 °C; $[\alpha]_D^{21} = -22$ (c = 1.2, ethanol); IR: $\tilde{\nu}$ 3421, 4104, 2922, 1740, 1710, 1407, 1368, 1202, 741, 722 cm^{-1}; MS (*m/z*) ESI$^+$: 380 [M−Cl]$^+$; ^{1}H NMR ($CDCl_3$): δ 2.00 (s, 3H, thienyl–C*H*$_3$), 2.05 (s, 3H, thienyl–C*H*$_3$), 2.30–2.44 (m, 1H, NCHC*H*$_2$), 2.58–2.66 (m, 2H, NCH$_2$C*H*$_2$), 2.78–2.86 (m, 1H, NCHC*H*$_2$), 3.39 (ddd, 1H, J = 12.4, 9.6, 6.6 Hz, NC*H*$_2$CH$_2$), 3.56 (ddd, J = 12.4, 9.6, 6.6 Hz, NC*H*$_2$CH$_2$), 3.65 (dd, 1H, J = 22.5, 13.9 Hz, NC*H*$_2$CHF), 3.99 (ddd, 1H, J = 34.0, 13.9, 3.6 Hz, NC*H*$_2$CHF), 4.68 (dd, 1H, J = 12.0, 7.0 Hz, NC*H*CO), 5.43 (dt, 1H, J = 52.2, 3.6 Hz, C*H*F), 6.04 (t, 1H, J = 7.3 Hz, =C*H*CH$_2$), 6.79 (d, 1H, J = 5.0 Hz, SC*H*), 6.93 (d, 1H, J = 5.0 Hz, SC*H*), 7.18 (d, 1H, J = 5.0 Hz, SC=C*H*), 7.39 (d, 1H, J = 5.0 Hz, SC=C*H*); $C_{19}H_{23}ClFNO_2S_2$ (415.97) 0.8CH_3OH: C 53.85, H 5.98, N 3.17, S 14.52; found. C53.60, H 6.24, N 3.01, S 14.20.2

(2*S*,4*S*)-**16b**: Gray crystals; yield: 87%; mp: 123–127 °C; $[\alpha]_D^{18} = -16$ (c = 0.94, ethanol); IR: $\tilde{\nu}$ 3410, 3096, 2969, 2492, 1746, 1636, 1372, 1201, 719 cm^{-1}; MS (*m/z*) ESI$^+$: 380 [M−Cl]$^+$, 402 [M−Cl+Na]$^+$; ^{1}H NMR (CD_3OD): δ 1.90 (s, 3H, thienyl–C*H*$_3$), 1.97 (s, 3H, thienyl–C*H*$_3$), 2.44–2.61 (m, 3H, 2H of NCH$_2$C*H*$_2$ and 1H of NCHC*H*$_2$), 2.78–2.91 (m, 1H, NCHC*H*$_2$), 3.26–3.47 (m, 3H, 2H of NC*H*$_2$CH$_2$ and 1H of NC*H*$_2$CHF), 3.89 (t, 1H, J = 14.5 Hz, NC*H*$_2$CHF), 4.45 (dd, 1H, J = 11.0, 4.6 Hz, NC*H*CO), 5.33 (d, 1H, J = 50.9 Hz, C*H*F), 5.93 (t, 1H, J = 7.3 Hz, =C*H*CH$_2$), 6.71 (d, 1H, J = 5.0 Hz, SC*H*), 6.84 (d, 1H, J = 5.0 Hz, SC*H*), 7.11 (d, 1H, J = 5.0 Hz, SC=C*H*),7.30 (d, 1H, J = 5.0 Hz, SC=C*H*); $C_{19}H_{23}ClFNO_2S_2$ (415.97) 0.8CH_3OH: C, 53.85; H, 5.98; N, 3.17; S, 14.52. Found: C, 53.60; H, 6.24; N, 3.01; S, 14.20.

(2*S*,4*R*)-**20a**: yellow foam; yield: 85%; $[\alpha]_D^{22} = -12$ (c = 0.85, ethanol); IR: $\tilde{\nu}$ 3022, 2927, 2855, 1732, 1444, 1149, 703 cm^{-1}; MS (*m/z*) ESI$^+$: 354 [M−Cl]$^+$; ^{1}H NMR (CD_3OD): δ 1.88–2.07 (m, 1H, NCHC*H*$_2$), 2.44–2.62 (m, 3H, 2H of NCH$_2$C*H*$_2$ and 1H of NCHC*H*$_2$), 2.75 (dd, 1H, J = 17.9, 6.0 Hz, C*H*$_2$COO), 2.85 (dd, 1H, J = 17.9, 6.0 Hz, C*H*$_2$COO), 3.18–3.25 (m, 1H, NC*H*$_2$CH$_2$), 3.47 (dd, 1H, J = 20.8, 14.4 Hz, NC*H*$_2$CHF), 3.54–3.61 (m, 1H, NC*H*$_2$CH$_2$), 3.68 (dd, 1H, J = 34.4, 14.4, 3.9 Hz, NC*H*$_2$CHF), 4.00–4.02 (m, 1H, NC*H*CH$_2$), 5.23 (dt, 1H, J = 52.2, 3.7 Hz, C*H*F), 5.99 (t, 1H, J = 7.5 Hz, =C*H*CH$_2$), 7.10–7.37 (m, 10H, H$_{aromat.}$); Calcd for $C_{22}H_{25}ClFNO_2$ (388.89) 0.4CH_3OH: C, 66.93; H, 6.66; N, 3.48. Found: C, 66.98; H, 6.37; N, 3.16.

(2*S*,4*S*)-**20a**: yellow foam; yield: 83%; $[\alpha]_D^{24} = -15$ (c = 0.76, ethanol); IR: $\tilde{\nu}$ 3419, 2924, 1729, 1408, 1196, 716 cm^{-1}; MS (*m/z*) ESI$^+$: 354 [M−Cl]$^+$; ^{1}H NMR (CD_3OD): δ 2.01 (ddd, 1H, J = 30.0, 15.1, 7.3 Hz, NCHC*H*$_2$), 2.49–2.78 (m, 5H, 1H of NCHC*H*$_2$, 2H of NCH$_2$C*H*$_2$ and 2H of C*H*$_2$COO), 2.89–2.94 (m, 1H, NC*H*$_2$CH$_2$), 3.01 (ddd, 1H, J = 34.8, 12.8, 4.1 Hz, NC*H*$_2$CHF), 3.49–3.60 (m, 3H, NC*H*CH$_2$, NC*H*$_2$CH$_2$ and NC*H*$_2$CHF), 5.25 (dt, 1H, J = 53.2, 4.6 Hz, C*H*F), 6.08 (t, 1H, J = 7.3 Hz, =C*H*CH$_2$), 7.19–7.45 (m, 10H, H$_{aromat.}$); Calcd for $C_{22}H_{25}ClFNO_2$ (388.89) 0.7CH_3OH: 67.34, H, 6.92; N, 3.46. Found: C, 67.46; H, 7.16; N, 3.08.

(2*S*,4*R*)-**20b**: gray foam; yield: 93%; $[\alpha]_D^{22} = -20$ (c = 0.95, ethanol), IR: $\tilde{\nu}$ 3101, 2923, 2855, 1737, 1425, 1029, 722 cm^{-1}; MS (*m/z*) ESI$^+$: 394 [M−Cl]$^+$; ^{1}H NMR (CD_3OD): δ 1.89 (s, 3H, thienyl–C*H*$_3$), 1.95 (s, 3H, thienyl–C*H*$_3$), 1.92–2.05 (m, 1H, NCHC*H*$_2$), 2.45–2.56 (m, 3H, 1H of NCHC*H*$_2$ and 2H of NCH$_2$C*H*$_2$), 2.69–2.78 (m, 2H, C*H*$_2$COO), 3.12 (dt, 1H, J = 12.5, 7.3 Hz, NC*H*$_2$CH$_2$), 3.38 (dd, 1H, J = 22.5, 13.7 Hz, NC*H*$_2$CHF), 3.47 (dt, 1H, J = 12.5, 8.0 Hz, NC*H*$_2$CH$_2$), 3.76 (ddd, 1H, J = 33.7, 13.7, 3.8 Hz, NC*H*$_2$CHF), 3.86–3.92 (m, 1H, NC*H*CH$_2$), 5,25 (br d, 1H, J = 52.7 Hz, C*H*F), 5.95 (t, 1H, J = 7.3 Hz, =C*H*CH$_2$), 6.69 (d, 1H, J = 5.5 Hz, SC*H*), 6.82 (d, 1H, J = 5.0 Hz, SC*H*), 7.07 (d, 1H, J = 5.5 Hz, SC=C*H*), 7.28 (d, 1H, J = 5.0 Hz, SC=C*H*); Calcd for $C_{20}H_{25}ClFNO_2S_2$ (430.01) 0.9CH_3OH: C, 54.70; H, 6.28; N, 3.05; S, 13.98. Found: C, 54.42; H, 6.05; N, 2.88; S, 13.81.

(2*S*,4*S*)-**20b**: gray foam; yield: 85%; $[\alpha]_D^{24} = -24$ (c = 0.83, ethanol); IR: $\tilde{\nu}$ 3440, 3100, 2945, 2560, 1726, 1457, 1171, 718 cm^{-1}; MS (*m/z*) ESI$^+$: 394 [M−Cl]$^+$; ^{1}H NMR (CD_3OD): δ 1.91–2.02 (m, 1H, NCHC*H*$_2$), 1.99 (s, 3H, thienyl–C*H*$_3$), 2.04 (s, 3H, thienyl–C*H*$_3$), 2.50–2.75 (m, 5H, 2H of NCH$_2$C*H*$_2$, 2H of C*H*$_2$COO and 1H of NCHC*H*$_2$), 2.80–2.86 (m, 1H, NC*H*$_2$CH$_2$), 2.97 (ddd, 1H, J = 34.8, 12.8, 4.1 Hz, NC*H*$_2$CHF), 3.38–3.46 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*CH$_2$), 3.65 (dd, 1H, J = 18.6, 12.8 Hz, NC*H*$_2$CHF), 5.24 (dt, 1H, J = 53.2, 5.0 Hz, C*H*F), 6.05 (t, 1H, J = 7.3 Hz,=C*H*CH$_2$), 6.78 (d, 1H, J = 5.0 Hz, SC*H*), 6.90 (d, 1H, J = 5.0 Hz, SC*H*), 7.15 (d, 1H, J = 5.0 Hz, SC=C*H*), 7.35 (d, 1H, J = 5.0 Hz, SC=C*H*), Calcd for $C_{20}H_{25}ClFNO_2S_2$ (430.01) 0.5CH_3OH: C, 55.20; H, 6.10; N, 3.14; S, 14.91. Found: C, 55.47; H, 5.95; N, 2.83; S, 14.67.

5.7. Bioassay

D8 cells, grown in RMP I1640 medium (Gibco-BRL Life Technologies) containing 10% FBS (Gibco-BRL Life Technologies) to near confluence in 48-well tissue culture plates (Costar) (approximately 60,000 cells per well in 48-well plates), were rinsed once with phosphate buffered saline (PBS) and pre-incubated in 100 μL Hanks, balanced salt solution (HBSS) for 10 min at room temperature. [^{3}H] GABA (Amersham Pharmacia Biotech) was added to final concentrations of 151 nM. The cells were incubated for 20 min at room temperature. The reaction was terminated by aspiration of the HBSS and the cells were washed three times rapidly (10 s/wash) with cold PBS. The cells were then solubilized in 2 N NaOH and an aliquot was measured by liquid scintillation counting

(Beckman LS 5000 TA) to quantify the uptake of [^{3}H] GABA. Inhibition studies were performed by adding inhibitor at the beginning of the transport assay and IC_{50} values were determined by nonlinear regression analysis.

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