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Original article

Phenyl acetate derivatives, fluorine-substituted on the phenyl group, as rapid recovery hypnotic agents with reflex depression

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ABSTRACT

We report the synthesis of novel, potentially hypnotic fluorine-substituted phenyl acetate derivatives. We describe the structure–activity relationship that led us to the promising derivative: ethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-5-ethoxy-2-fluorophenyl) acetate (**55**). The unique pharmacological features of compound **55** are its relatively high affinity for the $GABA_A$ receptor, together with a unique affinity for the NMDA receptor, different to propanidid and AZD3043. In animal models, compound **55** showed stronger hypnotic potency and longer duration of LORR than propanidid and AZD3043, but also maintained a rapid recovery time to walking and behavioral recovery. In particular, compound **55** displayed reflex depression during infusion.

1. Introduction

"Soft" drugs are those molecules that are purposefully designed to be rapidly metabolized (metabolically labile) [1]. In anesthesia, a soft drug is useful because it enables precise titration to effect and rapid recovery, which may allow swift and clear-headed recovery of consciousness and early home readiness [2].

Propofol (Fig. 1) has been associated with long-delayed awakening after prolonged infusion [3], which may mean that several *days* are required for complete dissipation of the drug's effects.

The role of fluorine in medicinal chemistry in recent years has been remarkable [4]. Drug candidates with one or more fluorine atoms have become commonplace [5]. The chief advantage of fluorine, especially as trifluoromethyl- or fluoro-substituted aryl compounds, is that it imparts a variety of properties to medicines, including increased lipid solubility and improved drug transport across the blood–brain barrier [6].

In our previous work [7], we introduced fluorine atoms to the ether (R_1) and ester (R_2) moieties of the parent structure of propanidid. Among the fluorine-containing phenyl acetate derivatives, compound **1** showed a shorter duration of 'loss of righting reflex' (LORR) and faster recovery time (time to walking and time to behavioral recovery) than propanidid or AZD3043. This rapid recovery might make compound **1** suitable as a soft drug for precise titration and allow swift and clear-headed recovery of consciousness and early home readiness.

However, there has been controversy about soft drugs, such as AZD3043 and MOC-etomidate, and whether they offer too rapid a recovery from anesthesia. Relatively longer durations of LORR could reduce the dose administered and still be manipulated readily in the clinic [2,17]. We tested compounds with fluorine-containing alkyl groups at R_2 and they had a shorter duration of LORR than propanidid and AZD3043 because of the faster metabolism by an esterase (compound **1**). The faster metabolism may have been due to the improved lipid solubility of the ester moiety (R_2). However, a fluorine atom on the aryl group was at a relatively long distance and would be expected to have little influence on the ester moiety. Thus, we proposed that compounds fluoro-substituted on the aryl group would show longer durations of LORR compared with the introduction of fluorine on the alkyl group on the ester moiety (R_2). Based on animal model tests, we confirmed our hypothesis that the optimized compounds showed longer durations of LORR and, at the same time, maintained rapid recoveries.

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E-mail address: gszhang@mail.hust.edu.cn (G. Zhang).

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0223-5234/

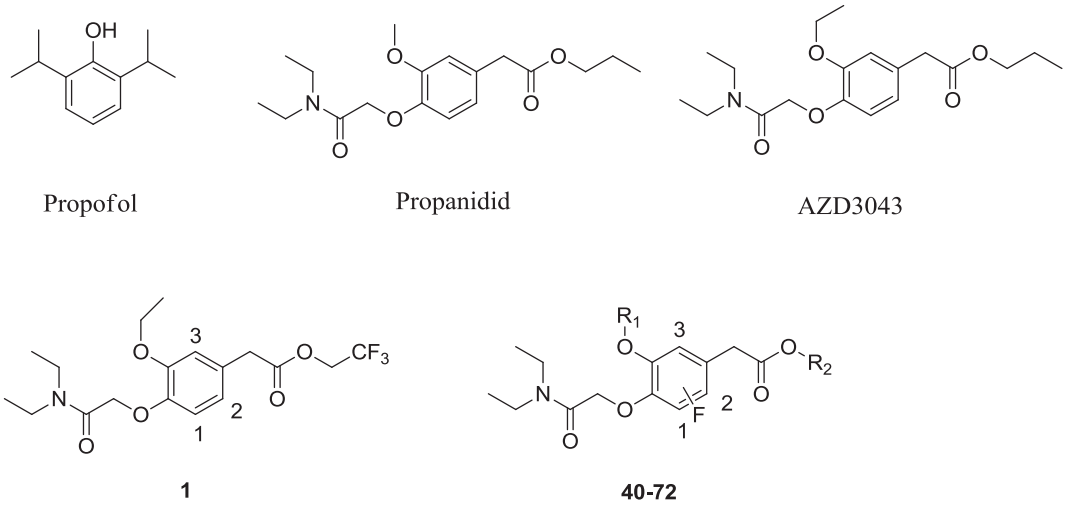


Fig. 1. Title and reference compounds.

2. Chemistry

Synthesis of the novel fluorine-containing phenyl acetate derivatives was performed according to the reaction pathway illustrated in Scheme 1. Compounds **40**–**72** were prepared by a six-step reaction. Propanidid, AZD34043, and propofol were also synthesized.

First, 2-bromo-fluorophenols (**2**) were reacted with haloalkanes to provide 2-bromofluorophenyl ethers (**3**). Alkoxy fluorophenols (**4**) were obtained from **3** under *n*-BuLi, H_2O_2, acetic acid and −78 °C conditions [8]. Subsequently, **4** and glyoxylic acid (50% aqueous solution) were converted to the corresponding 2-OH-fluorophenyl acetic acid derivatives (**5**) in 80–90% overall yield according to previous synthetic procedures used for similar compounds [9]. Intermediate phenyl acetic acid derivatives (**6**) were prepared from the reduction of **5** with dihydrate stannous chloride, which were further esterified with various ethanols to afford **7**–**39**. The corresponding esters were reacted with 2-chloro-N,N-diethylacetamide to obtain the final title compounds **40**–**72**.

3. Pharmacology

3.1. In vitro studies

The new compounds were all dissolved in 5% DMSO. The following specific radioligands and tissue sources were used: (a) $GABA_A$ receptor, [^{3}H]EBOB, rat frontoparietal cortex [10], (b) NMDA receptor, [^{3}H]MK-801, cerebral cortex [11], (c) serotonin 5-HT_{1A} receptor, [^{3}H]8-OH-DPAT, rat brain cortex [12], (d) serotonin 5-HT_{2A} receptor, [^{3}H]ketanserin, rat brain cortex [12], (e) serotonin 5-HT_{2C} receptor, [^{3}H]mesulergine, rat brain cortex [12], (f) 5-HTT transporter, [^{3}H]paroxetine, rat cerebral cortex [13], (g) dopamine D_2 receptor, [^{3}H]spiperone, rat striatum [12], (h) dopamine D_3 receptor, [^{3}H]7-OH-DPAT, rat olfactory tubercle [14], (i) histamine H_1 receptor, [^{3}H]mepyramine, guinea pig cerebellum [15], (j) adrenergic α_1 receptor, [^{3}H]prazosin, rat brain cortex [16], and (k) adrenergic α_2 receptor, [^{3}H]rauwolscine, rat brain cortex [16].

Total binding was determined in the absence of non-specific binding and compounds. Specific binding was determined in the presence of compounds. Non-specific binding was determined as the difference between total and specific binding.

$$\text{Percentage of inhibition}(\%) = (\text{total binding} - \text{specific binding}) \times 100\% / (\text{total binding} - \text{nonspecific binding})$$

Blank experiments were carried out to determine the effect of 5% DMSO on the binding; no effect was observed. Compounds were tested at least three times at 50 μM.

3.2. In vivo studies

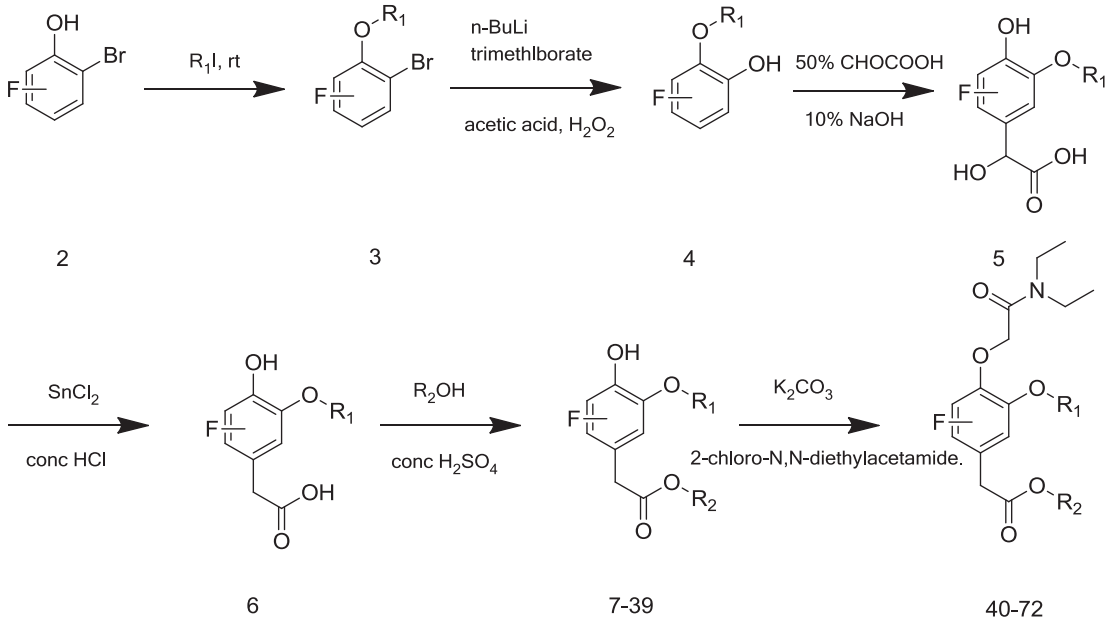
Hypnotic activities of compounds were evaluated *in vivo* in animal models. HD_{50}, Therapeutic Index (TI), duration of LORR, and recovery time (time to walking and time to behavioral recovery) were tested in a rat model.

4. Results and discussion

4.1. Hypnotic activity

In our previous study, the compounds with a fluorine-containing alkyl group on R_1 showed decreased hypnotic potency. Thus the fluorine-containing alkyl groups were not selected for R_1. The results showed that the compounds with fluorine substituted at the 2-position of the phenyl group and an Et group at R_1 displayed stronger hypnotic potencies than propanidid (HD_{50} = 7.9 mg kg^{-1}) and AZD3043 (HD_{50} = 8.5 mg kg^{-1}) in rat (Table 1). The HD_{50} values of compounds **55**, **56**, and **57** were 5.6, 5.3, and 4.8 mg kg^{-1}, respectively. However, compounds **40**–**49** with fluorine substituted at the 1-position of the phenyl group showed markedly decreased hypnotic potencies. Compounds **60**–**69** with fluorine substituted at the 3-position of the phenyl group showed further decreased hypnotic potencies.

These results suggested the importance of the fluoro-substituted position for the hypnotic activities of compounds synthesized. The alkyl group on R_1 had a marked influence on the hypnotic potency of compounds with fluorine at the 2-position of the phenyl group. Compounds **55**–**59** with Et at R_1 showed largely decreased hypnotic potency (5.6, 5.3, 4.8, 9.8, and 11.6 mg kg^{-1}, respectively), compared with compounds **50**–**54** with Me at R_1 (10.0, 15.8, 11.5, 18.4, and 15.6 mg kg^{-1}, respectively). However, fluorine at the 1- or 3-position of the phenyl group had little influence on the hypnotic potency of the compounds.



Scheme 1.

Regarding the R_2 group, compounds with an alkyl group showed greater hypnotic potency than those with fluorine-containing alkyl groups, regardless of the fluoro-substituted position.

To further assess the structure–activity relationship of hypnotic potency, based on the structure of compound **57**, we introduced larger groups (longer-chain alkyl groups) at R_1 and R_2. We introduced *n*-Pr at R_1 or *n*-Bu and *s*-Bu at R_2, but there was no improvement in hypnotic potency after these modifications (Table 2).

Regarding the duration of LORR, among all of the fluorine-substituted derivatives synthesized, we found that compounds fluoro-substituted at the 2-position of the phenyl group showed markedly prolonged duration of LORR. Compounds **55**, **56**, and **57** (209.6, 215.3, and 226.2 s, respectively) showed considerably longer durations of LORR than propanidid and AZD3043 (93.3 and 74.2 s). Compounds fluoro-substituted at the 1- or 3-position of the phenyl group also showed longer durations of LORR with propanidid and AZD3043. Although the durations of LORR of compounds **55**, **56**, and **57** were prolonged, the rapid recoveries to walking and to behavioral recovery were maintained, compared with propofol (time to walk: 286.5 s, time to behavioral recovery: 335.8 s; Fig. 2), particularly compound **55** (time to walk: 59.8 s, time to behavioral recovery: 68.4 s).

4.2. Hypnosis in response to bolus administration

Compound **55** (11.2, 16.8, and 22.4 mg kg^{-1}; $n = 4$ for each dose), compound **56** (10.6, 15.9, and 21.2 mg kg^{-1}; $n = 4$ for each dose), compound **57** (9.6, 14.4, and 19.2 mg kg^{-1}; $n = 4$ for each dose), propanidid (17.0, 25.5, and 34.0 mg kg^{-1}; $n = 4$ for each dose), AZD3043 (15.8, 23.7, and 31.6 mg kg^{-1}; $n = 4$ for each dose), and propofol (5.3, 10.6, and 15.9 mg kg^{-1}; $n = 4$ for each dose) all showed dose-dependent LORR after IV bolus administration. For each compound, onset of hypnosis was similarly rapid (<10 s from completion of injection). The slopes of dose–response curves of compounds **55**, **56**, and **57** were shallower than that of propofol (Fig. 3).

4.3. Hypnosis in response to i.v. infusion

In 20-min, 1-h, and 3-h infusion tests in rats, the durations of LORR of compound **55** were shorter than those of propofol. Although the recovery time to walking with compound **55** was prolonged compared with propanidid and AZD3043, it was considerably shorter than that of propofol (Fig. 4). In 20-min, 1-h, and 3-h infusions, rapid recovery with compound **55** was maintained and was largely unaffected by the duration of the infusion.

4.4. Reflex depression

When we tested the hypnotic activities of fluorine-containing phenyl acetate derivatives in rat, we found that compound **55** had reflex depression effects during the duration of LORR. We compared compound **55** with propanidid and AZD3043 in terms of reflex depression effect. After 2 × HD_{50} administration of each compound, compound **55** had obvious reflex depression before 120 s (Table 3), whereas propanidid and AZD3043 did not.

4.5. In vitro radioligand binding

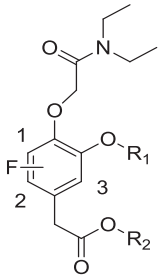
In the *in vitro* radioligand-binding test, compounds were tested at least three times at 50 μM. Compound **55** (89.2%) showed greater binding affinity than propanidid (70.2%) and AZD3043 (83.2%; Table 4). To investigate the off-target activities of drug candidate **55**, we tested its binding profile to $GABA_A$, NMDA, dopamine D_2, D_3, H_1, $5HT_{1A}$, $5HT_{2A}$, $5HT_{2C}$, α_1 and α_2 receptors, and the 5HTT transporter. Compound **55** displayed some NMDA receptor binding (56.6%; Fig. 5), which was not seen with propanidid, AZD3043, or other fluorine-containing phenyl acetate derivatives.

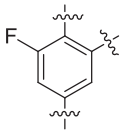
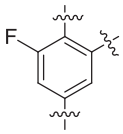
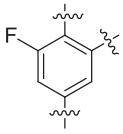
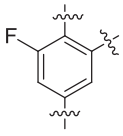
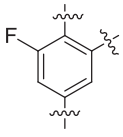
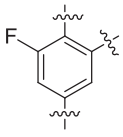
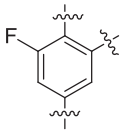
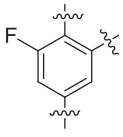
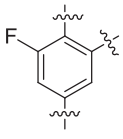
5. Conclusions

We introduced fluorine to the phenyl group of the propanidid framework. Fluorine at the 1- and 3-positions of the phenyl group decreased the hypnotic potencies, regardless of the ether (R_1) and ester (R_2) residues. However, we found some unexpected results with fluorine at the 2-position of the phenyl group. Compounds **55**, **56**, and **57** with an Et group at R_1 showed longer durations of LORR than propanidid and AZD3043, but maintained the rapid recovery time to walking and behavioral recovery.

There has been controversy as to whether 'soft' drugs, such as AZD3043 and MOC-etomidate, offer too rapid a recovery from

Table 1
Hypnotic activity of fluorine-substituted phenyl acetate derivatives in rats.



Compound	F	R_1	R_2	HD_{50} (mg kg^{-1})[a]	LD_{50} (mg kg^{-1})[a]	TI	Duration of LORR (s)
40		Me	Et	17.2 (15.8–18.3)	239.1 (234.2–242.5)	13.9	72.0 ± 23.5
41		Me	*n*-Pr	16.8 (14.2–17.6)	188.2 (185.5–194.3)	11.2	68.9 ± 14.5
42		Me	*i*-Pr	14.2 (13.5–15.8)	176.1 (174.6–178.5)	12.4	79.5 ± 6.8
43		Me	CH_2CF_3	16.9 (14.5–18.4)	164.6 (159.2–166.6)	9.8	71.1 ± 5.3
44		Me	$CH_2(CH_2)CF_3$	15.4 (14.7–17.8)	147.8 (144.5–149.8)	9.6	84.2 ± 8.6
45		Et	Et	18.5 (17.6–21.3)	223.9 (218.2–225.3)	12.1	66.5 ± 6.6
46		Et	*n*-Pr	14.8 (11.2–15.3)	174.6 (168.6–178.3)	11.8	76.6 ± 9.8
47		Et	*i*-Pr	19.5 (17.9–22.4)	255.5 (252.2–261.8)	13.1	92.7 ± 11.8
48		Et	CH_2CF_3	14.6 (13.8–15.8)	127.1 (125.6–129.7)	8.7	76.6 ± 6.7

(*continued on next page*)

Table 1 (*continued*)

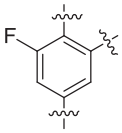
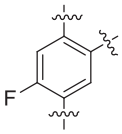
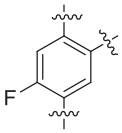
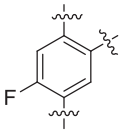
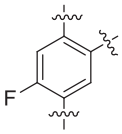
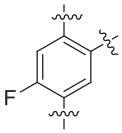
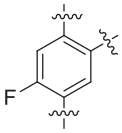
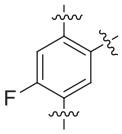
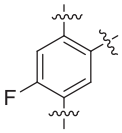
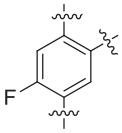
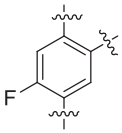
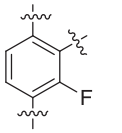
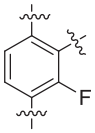
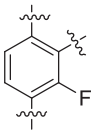
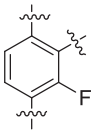
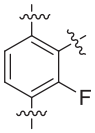
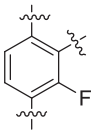
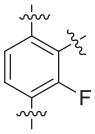
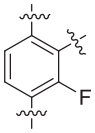
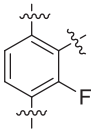
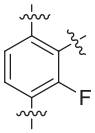
Compound	F	R_1	R_2	HD_{50} (mg kg^{-1})[a]	LD_{50} (mg kg^{-1})[a]	TI	Duration of LORR (s)
49		Et	$CH_2(CH_2)CF_3$	18.3 (17.7–21.2)	181.2 (177.5–182.4)	9.9	88.8 ± 8.7
50		Me	Et	10.0 (9.8–11.4)	113.0 (111.1–114.4)	11.3	82.2 ± 5.6
51		Me	*n*-Pr	15.8 (14.8–16.9)	199.1 (197.1–201.6)	12.6	79.3 ± 6.7
52		Me	*i*-Pr	11.5 (10.5–13.2)	155.3 (151.3–157.9)	13.5	86.6 ± 8.5
53		Me	CH_2CF_3	18.4 (16.7–20.1)	171.1 (168.6–172.4)	9.3	75.4 ± 7.5
54		Me	$CH_2(CH_2)CF_3$	15.6 (14.2–16.8)	151.3 (149.5–153.6)	9.7	88.9 ± 9.9
55		**Et**	**Et**	**5.6 (5.2–5.8)**	**74.5 (71.9–75.8)**	**14.4**	**209.6 ± 30.1**
56		**Et**	***n*-Pr**	**5.3 (4.9–5.4)**	**70.5 (68.6–71.2)**	**13.3**	**215.3 ± 37.8**
57		**Et**	***i*-Pr**	**4.8 (4.2–5.0)**	**60.9 (58.4–62.3)**	**12.7**	**226.2 ± 34.4**
58		Et	CH_2CF_3	9.8 (8.9–11.2)	112.7 (109.3–113.8)	11.5	186.1 ± 45.6
59		Et	$CH_2(CH_2)CF_3$	11.6 (11.4–12.9)	124.1 (122.6–126.3)	10.7	238.3 ± 56.5
60		Me	Et	21.6 (19.7–22.1)	200.9 (198.8–202.2)	9.3	89.8 ± 9.3

Table 1 (*continued*)

Compound	F	R_1	R_2	HD_{50} (mg kg^{-1})[a]	LD_{50} (mg kg^{-1})[a]	TI	Duration of LORR (s)
61		Me	*n*-Pr	19.4 (18.4–21.2)	168.8 (166.3–169.4)	8.7	76.6 ± 8.6
62		Me	*i*-Pr	17.9 (16.5–18.8)	141.4 (139.6–143.2)	7.9	91.4 ± 10.1
63		Me	CH_2CF_3	22.8 (20.9–23.2)	184.7 (182.4–185.9)	8.1	88.7 ± 8.8
64		Me	$CH_2(CH_2)CF_3$	21.4 (20.7–22.8)	158.4 (156.6–159.9)	7.4	92.6 ± 10.3
65		Et	Et	19.8 (18.6–20.6)	200.0 (199.3–203.2)	10.1	77.7 ± 7.9
66		Et	*n*-Pr	16.6 (15.5–17.9)	172.6 (170.7–174.1)	10.4	81.4 ± 8.3
67		Et	*i*-Pr	17.9 (16.1–18.3)	164.7 (162.5–166.6)	9.2	102.9 ± 11.1
68		Et	CH_2CF_3	21.1 (19.8–22.4)	162.5 (161.1–164.4)	7.7	83.3 ± 9.6
69		Et	$CH_2(CH_2)CF_3$	24.6 (22.3–25.2)	182.1 (180.7–183.8)	7.4	89.8 ± 10.2
AZD3043				7.9 (7.7–9.1)	82.8 (79.6.2–83.9)	10.1	74.2 ± 10.5
Propanidid				8.5 (7.8–10.4)	80.6 (78.8–82.2)	9.6	93.3 ± 16.1
Propofol				5.3 (4.5–5.7)	30.7 (26.9–31.3)	5.9	349.3 ± 93.3

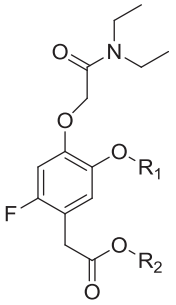
Data are expressed as means ± SD ($n = 10$).
The compounds with excellent hypnotic activities were bolded.
[a] 95% confidence limits.

anesthetic drug effects. Concern has mounted about perioperative awareness due to inadequate delivery of *i.v.* anesthesia [2]. Compound **55** might be a better choice, based on this.

Compound **55** showed NMDA receptor binding and a reflex depression effect, which were not seen with propanidid, AZD3043, or other fluorine-containing phenyl acetate derivatives. The analgesic effect (reflex depression) might be associated with the NMDA receptor binding of compound **55**. Further experiments are needed to assess this.

In conclusion, compound **55** may be a promising hypnotic agent for further development because of its stronger hypnotic potency and prolonged duration of action, which could allow a reduced infusion dose compared with propanidid and AZD3043. Compound **55** also shows more rapid recovery time to walking and behavioral

Table 2
Hypnotic activities of fluorine substituted phenyl acetate derivatives.



Compound	R_1	R_2	HD_{50} (mg kg^{-1})[a]	LD_{50} (mg kg^{-1})[a]	TI	Duration of LORR (s)
57	Et	i-Pr	4.8 (4.2–5.0)	60.9 (58.4–62.3)	12.7	226.2 ± 34.4
70	Et	*n*-Bu	9.7 (9.4–10.3)	108.6 (106.5–109.8)	11.2	158.3 ± 31.5
71	Et	*i*-Bu	8.4 (8.1–8.6)	90.7 (88.8–92.4)	10.8	145.3 ± 29.6
72	*n*-Pr	*i*-Pr	10.6 (9.8–11.1)	101.8 (99.9–104.6)	9.6	133.9 ± 28.9

[a] 95% confidence limits. Data was expressed as means ± SD ($n = 10$).

recovery than propofol, enabling its precise titration to effect and rapid recovery. The reflex depression effect of compound **55** may also decrease the use of analgesic drugs during anesthesia.

6. Experimental

6.1. Chemistry experimental

Melting points were determined in open capillary tubes and are uncorrected. NMR spectra are recorded at 400 MHz on a Varian Inova Unity 200 spectrometer in $CDCl_3$ and DMSO-d_6 solution. Chemical shifts are given in δ values (ppm), using tetramethylsilane (TMS) as the internal standard; coupling constants (J) were given in Hz. Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signal). Reagents were all of analytical grade or of chemical purity. Analytical TLC was performed on silica gel GF254. Column chromatographic purification was carried out using silica gel. Compound purity is determined by high performance liquid chromatography (HPLC), and all tested compounds were >95% purity. HRMS experiments were performed with TripleTOF 5600 (AB SCIEX). HPLC methods used the following: Agilent 1100 spectrometer; column, Shim-pack ODS 5.0 μm × 150 mm × 2.0 mm I.D (SHIMADZU, Japanese); mobile phase, 0.0167% HCOOH (TEDIA Company, USA)/acetonitrile (Merck Company, Germany) 50/50; flow rate, 0.2 ml/min; column temperature, 40 °C; UV detection was performed at 254 nm.

6.1.1. Synthesis of different fluoro-substituted phenylate derivatives

To a solution of 2-bromo-4-fluorophenol (0.1 mol) in DMF (100 ml) was added K_2CO_3 (0.1 mol) and ethyl iodide (methyl iodide or propyl iodide) (0.1 mol) stirred 24 h at room temperature. Water (400 ml) was added and the mixture was extracted with hexane. The organic phase was washed with water, dried, filtered over a plug of silica gel and evaporated to yield the objected compound.

*6.1.1.1. 2-Bromo-1-fluoro-3-methoxybenzene (**3a**).* Yellow oil; Yield: 90%; ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3H), 6.68–6.81 (m, 2H), 7.18–7.30 (m, 1H). MS (ESI): 205.1.

*6.1.1.2. 2-Bromo-4-fluoro-1-methoxybenzene (**3b**).* Yellow oil; Yield: 87%; ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H), 6.87–7.29 (m, 3H). MS (ESI): 205.1.

*6.1.1.3. 1-Bromo-3-fluoro-2-methoxybenzene (**3c**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 3H), 6.88–7.26 (m, 3H). MS (ESI): 205.1.

*6.1.1.4. 2-Bromo-1-ethoxy-3-fluorobenzene (**3d**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, $J = 7.02$ Hz), 4.03–4.09 (m, 2H), 6.83–7.31 (m, 3H). MS (ESI): 219.1.

*6.1.1.5. 2-Bromo-1-ethoxy-4-fluorobenzene (**3e**).* Yellow oil; Yield: 83%; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H, $J = 7.12$ Hz), 4.02–4.08 (m, 2H), 6.85–7.28 (m, 3H). MS (ESI): 219.1.

*6.1.1.6. 1-Bromo-2-ethoxy-3-fluorobenzene (**3f**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, $J = 7.08$ Hz), 4.01–4.07 (m, 2H), 6.89–7.27 (m, 3H). MS (ESI): 219.1.

*6.1.1.7. 2-Bromo-4-fluoro-1-propoxybenzene (**3g**).* Yellow oil; Yield: 86%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H), 1.74–1.79 (m, 2H), 4.09 (t, 2H, $J = 6.98$ Hz), 6.86–7.29 (m, 3H). MS (ESI): 233.1.

6.1.2. Synthesis of different fluoro-substituted phenol derivatives

The 2-bromo-1-ethoxy-4-fluoro-benzene (0.1 mol) was dissolved in THF (25 ml). The solution was cooled to −75 °C and a solution of *n*-BuLi (9.73 ml, 2.4 M in hexane) was added slowly. The mixture was stirred for 30 min at −75 °C. Trimethylborate (0.1 mol) was added within 5 min. Acetic acid (0.1 mol) and a 30% solution of H_2O_2 (0.1 mol) were added sequentially. The mixture was stirred for 30 min at 0 °C and for 3 h at room temperature. Water was added and the mixture was extracted with ethyl acetate. The crude product was purified by column chromatography to yield the objected compound.

*6.1.2.1. 2-Fluoro-6-methoxyphenol (**4a**).* Yellow oil; Yield: 75%; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H), 5.40 (s, 1H), 6.64–6.78 (m, 3H). MS (ESI): 143.1.

*6.1.2.2. 5-Fluoro-2-methoxyphenol (**4b**).* Yellow oil; Yield: 82%; ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H), 5.82 (s, 1H), 6.56–6.76 (m, 3H). MS (ESI): 143.1.

*6.1.2.3. 3-Fluoro-2-methoxyphenol (**4c**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H), 5.80 (s, 1H), 6.61–6.82 (m, 3H). MS (ESI): 143.1.

*6.1.2.4. 2-Ethoxy-6-fluorophenol (**4d**).* Yellow oil; Yield: 78%; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H), 4.02–4.09 (m, 2H), 5.76 (s, 1H), 6.66–6.78 (m, 3H). MS (ESI): 157.1.

*6.1.2.5. 2-Ethoxy-5-fluorophenol (**4e**).* Yellow oil; Yield: 86%; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 4.03–4.10 (m, 2H), 5.73 (s, 1H), 6.65–6.79 (m, 3H). MS (ESI): 157.1.

*6.1.2.6. 2-Ethoxy-3-fluorophenol (**4f**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 4.02–4.11 (m, 2H), 5.73 (s, 1H), 6.62–6.80 (m, 3H). MS (ESI): 157.1.

*6.1.2.7. 5-Fluoro-2-propoxyphenol (**4g**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H), 1.73–1.79 (m, 2H), 4.08 (t, 2H, $J = 6.98$ Hz), 5.73 (s, 1H), 6.78–7.32 (m, 3H). MS (ESI): 171.1.

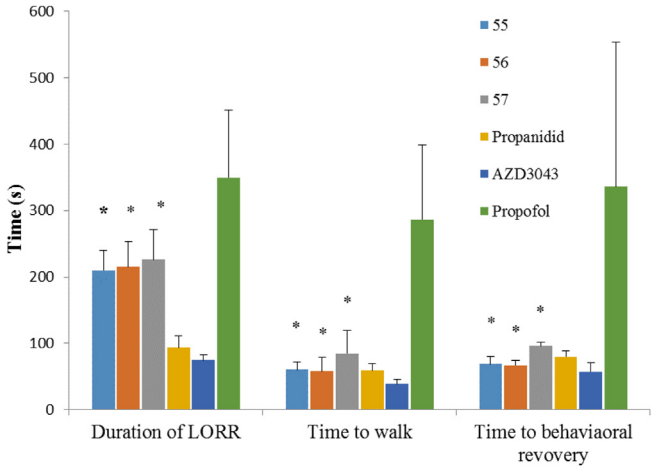


Fig. 2. Duration of the LORR and recovery time after 2 × HD_{50} administration of propofol, propanidid, AZD3043, and compounds **55**, **56**, and **57** in rat. Data are expressed as means ± SD (n = 10). $^{*}P < 0.05$, vs. propofol, one-way ANOVA with Dunnett's *post hoc* test.

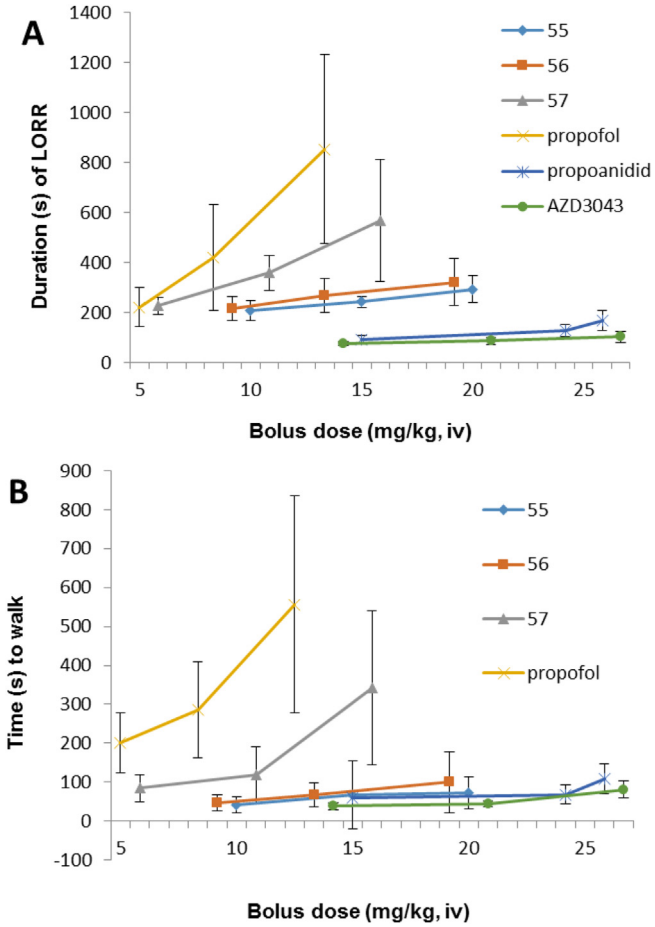


Fig. 3. Duration of LORR and recovery time to walking after bolus *i.v.* administration in rats. (A) Duration of LORR. (B) Time to walking. Compound **55** (11.2, 16.8, and 22.4 mg kg^{-1}; n = 4 for each dose), compound **56** (10.6, 15.9, and 21.2 mg kg^{-1}; n = 4 for each dose), compound **57** (9.6, 14.4, and 19.2 mg kg^{-1}; n = 4 for each dose), propanidid (17.0, 25.5, and 34.0 mg kg^{-1}; n = 4 for each dose), AZD3043 (15.8, 23.7, and 31.6 mg kg^{-1}; n = 4 for each dose), and propofol (5.3, 10.6, and 15.9 mg kg^{-1}; n = 4 for each dose). Data are expressed as means ± SD.

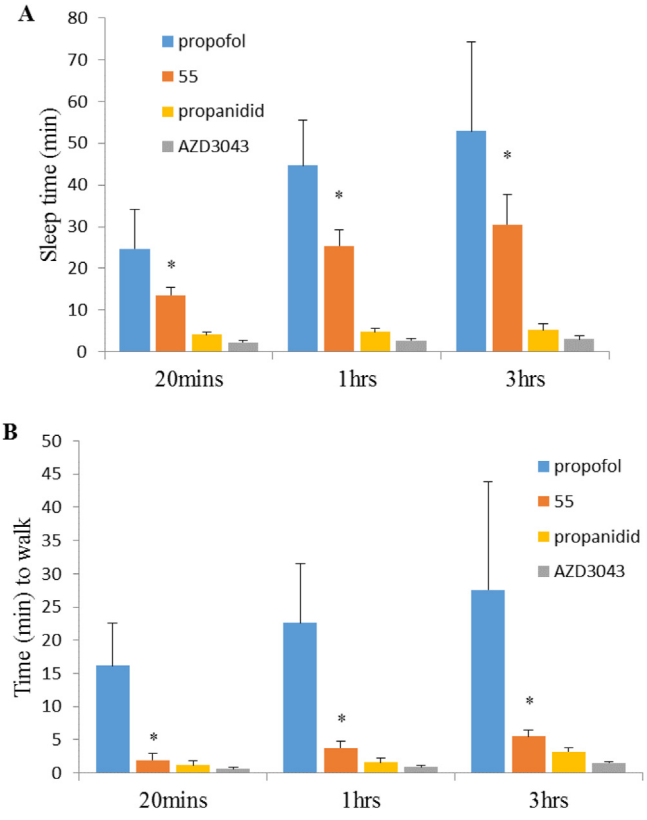


Fig. 4. Durations of the LORR and recovery times to walking of propofol, propanidid, AZD3043, and compound **55** in rats after a 20-min, 1-h, and 3-h *i.v.* infusion. (A) Duration of LORR. (B), Time to walking. Induction of hypnosis in rats was achieved using 2 × HD_{50} dose of the test compound, and immediately after induction, infusion via the tail vein was commenced at the HD_{50} dosage per min. Data are expressed as means ± SD. Propofol (5.3 mg kg^{-1} min^{-1}; n = 4, 4, or 3, respectively), propanidid (8.5 mg kg^{-1} min^{-1}; n = 5, 4, or 5, respectively), AZD3043 (7.9 mg kg^{-1}·min^{-1}; n = 5, 4, or 5, respectively), and compound **55** (5.6 mg kg^{-1} min^{-1}; n = 5, 4, or 5, respectively) in rats. $^{*}P < 0.05$, vs. propofol, one-way ANOVA with Dunnett's *post hoc* test.

Table 3
The effect of compound **55**, propanidid and AZD3043 on reflex depression in rat.

Compound	Duration of LORR (s)	Reflex depression[a] Time following injection (s)					
		10	20	30	60	120	180
55	209.6 ± 30.1	0	0	4	32	38	40
Propanidid	93.3 ± 16.1	34	40				
AZD3043	74.2 ± 10.5	36	40				

[a] Combined scores for each group of 10 rats, the response being graded from 0 (no reflex response) to 4 (full reflex response) for each rat. 2 × HD_{50} administration of each compound.

6.1.3. *Synthesis of 2-hydroxyphenylacetic acid derivatives*

A 10% sodium hydroxide solution (300 ml) was added slowly to the rapidly stirring solution of the appropriate phenol (**4a**–**g**) (0.4 mol), 50% glyoxylic acid (0.396 mol) and 100-ml distilled water at 0 °C. The reaction mixture was stirred at room temperature overnight and washed three times with ethyl acetate. The aqueous phase was adjusted to pH 3 using concentrated hydrochloric acid and extracted three times using ethyl acetate. The combined organic phases were evaporated under reduced pressure to obtain the final products.

Table 4
Chemical data of fluorine substituted phenyl acetate derivatives.

Compound	Octanol:water partition[a] (coefficient ± SD)	$GABA_A$ binding[a] (50 μmol) (mean ± SD)
40	102 ± 22	46.5 ± 3.9
41	218 ± 45	58.9 ± 4.6
42	185 ± 36	64.2 ± 6.8
43	322 ± 45	61.2 ± 3.9
44	645 ± 99	52.3 ± 4.4
45	260 ± 66	42.3 ± 3.6
46	427 ± 84	56.9 ± 4.2
47	288 ± 56	48.6 ± 2.5
48	603 ± 78	59.7 ± 5.4
49	1259 ± 189	55.6 ± 4.9
50	118 ± 24	68.9 ± 5.5
51	227 ± 49	44.2 ± 4.5
52	156 ± 32	48.3 ± 6.8
53	311 ± 43	56.8 ± 5.3
54	875 ± 111	66.7 ± 7.9
55	**369 ± 77**	**89.2 ± 8.8**
56	528 ± 89	78.4 ± 6.9
57	388 ± 109	85.2 ± 7.9
58	788 ± 39	68.7 ± 5.5
59	1159 ± 228	71.2 ± 6.6
60	196 ± 48	9.6 ± 1.3
61	289 ± 42	8.6 ± 1.6
62	336 ± 79	7.9 ± 1.8
63	456 ± 62	11.3 ± 2.4
64	675 ± 75	14.5 ± 1.2
65	289 ± 52	14.6 ± 2.2
66	689 ± 96	15.2 ± 1.9
67	366 ± 76	17.6 ± 2.7
68	774 ± 59	18.3 ± 3.3
69	1259 ± 268	14.2 ± 2.3
70	1122 ± 228	56.7 ± 2.6
71	1072 ± 247	64.2 ± 6.8
72	891 ± 39	72.8 ± 5.7
Propanidid	394 ± 40	70.2 ± 5.8
AZD3043	466 ± 54	83.2 ± 7.4

The compounds with excellent hypnotic activities were bolded.
[a] Data are expressed as means ± SD with respect to the mean values from at least three separate studies.

*6.1.3.1. 2-(3-Fluoro-4-hydroxy-5-methoxyphenyl)-2-hydroxyacetic acid (**5a**).* Yield: 83%; mp: 103–105 °C. ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H), 4.91 (s, 1H), 6.77–6.84 (m, 2H), 12.18 (s, 1H). MS (ESI): 217.1.

*6.1.3.2. 2-(2-Fluoro-4-hydroxy-5-methoxyphenyl)-2-hydroxyacetic acid (**5b**).* Yield: 86%; mp: 81–83 °C. ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 3H), 5.10 (s, 1H), 6.56–6.94 (m, 2H), 12.29 (s, 1H). MS (ESI): 217.1.

*6.1.3.3. 2-(2-Fluoro-4-hydroxy-3-methoxyphenyl)-2-hydroxyacetic acid (**5c**).* Yield: 84%; mp: 91–93 °C. ^{1}H NMR (DMSO-d_6) δ 3.71 (s, 3H), 5.08 (s, 1H), 6.54–6.83 (m, 2H), 12.33 (s, 1H). MS (ESI): 217.1.

*6.1.3.4. 2-(3-Ethoxy-5-fluoro-4-hydroxyphenyl)-2-hydroxyacetic acid (**5d**).* Yield: 78%; mp: 104–105 °C. ^{1}H NMR (DMSO-d_6) δ 1.29 (t, 3H, *J* = 6.96 Hz), 3.43 (s, 2H), 3.92–3.97 (m, 2H), 5.08 (s, 1H), 6.58–6.94 (m, 2H), 12.29 (s, 1H). MS (ESI): 231.1.

*6.1.3.5. 2-(5-Ethoxy-2-fluoro-4-hydroxyphenyl)-2-hydroxyacetic acid (**5e**).* Yield: 88%; mp: 104–106 °C. ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3H, *J* = 7.07 Hz), 3.45 (s, 2H), 3.93–3.98 (m, 2H), 5.12 (s, 1H), 6.54–6.73 (m, 2H), 12.30 (s, 1H). MS (ESI): 231.1.

*6.1.3.6. 2-(3-Ethoxy-2-fluoro-4-hydroxyphenyl)-2-hydroxyacetic acid (**5f**).* Yield: 89%; mp: 79–81 °C. ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3H, *J* = 6.98 Hz), 3.45 (s, 2H), 3.93–3.98 (m, 2H), 5.12 (s, 1H), 6.56–6.92 (m, 2H), 12.31 (s, 1H). MS (ESI): 231.1.

*6.1.3.7. 2-(2-Fluoro-4-hydroxy-5-propoxyphenyl)-2-hydroxyacetic acid (**5g**).* Yield: 77%; mp: 85–87 °C. ^{1}H NMR (DMSO-d_6) δ 0.92 (t, 3H, *J* = 7.01 Hz), 1.74–1.79 (m, 2H), 4.09 (t, 2H, *J* = 7.12 Hz), 5.09 (s, 1H), 6.78–6.82 (m, 2H), 12.28 (s, 1H). MS (ESI): 245.1.

6.1.4. Synthesis of phenylacetic acid derivatives

2-Hydroxyphenylacetic acid (0.2 mol) was added to a solution of concentrated hydrochloric acid (120 ml) and dihydrate stannous chloride (0.4 mol). After refluxing for 4 h, the reaction was cooled to room temperature. The resulting solid was collected by filtration and washed with distilled water. The white solid was air dried to obtain the final compound.

*6.1.4.1. 2-(3-Fluoro-4-hydroxy-5-methoxyphenyl) acetic acid (**6a**).* Yield: 89%; mp: 86–88 °C. ^{1}H NMR (DMSO-d_6) δ 3.45 (s, 2H), 3.77 (s, 3H), 5.06 (s, 1H), 6.64–6.68 (m, 2H), 12.19 (s, 1H). MS (ESI): 201.1.

*6.1.4.2. 2-(2-Fluoro-4-hydroxy-5-methoxyphenyl) acetic acid (**6b**).* Yield: 89%; mp: 115–116 °C. ^{1}H NMR (DMSO-d_6) δ 3.43 (s, 2H), 3.73 (s, 3H), 5.08 (s, 1H), 6.56–6.86 (m, 2H), 12.28 (s, 1H). MS (ESI): 201.1.

*6.1.4.3. 2-(2-Fluoro-4-hydroxy-3-methoxyphenyl) acetic acid (**6c**).* Yield: 89%; mp: 104–106 °C. ^{1}H NMR (DMSO-d_6) δ 3.43 (s, 2H), 3.71 (s, 3H), 5.10 (s, 1H), 6.49–6.62 (m, 2H), 12.17 (s, 1H). MS (ESI): 201.1.

*6.1.4.4. 2-(3-Ethoxy-5-fluoro-4-hydroxyphenyl) acetic acid (**6d**).* Yield: 89%; mp: 108–110 °C. ^{1}H NMR (DMSO-d_6) δ 1.26 (t, 3H, *J* = 7.06 Hz), 3.45 (s, 2H), 3.92–3.98 (m, 2H), 5.24 (s, 1H), 6.56–6.84 (m, 2H), 12.29 (s, 1H). MS (ESI): 215.1.

*6.1.4.5. 2-(5-Ethoxy-2-fluoro-4-hydroxyphenyl) acetic acid (**6e**).* Yield: 89%; mp: 101–103 °C. ^{1}H NMR (DMSO-d_6) δ 1.28 (t, 3H, *J* = 6.96 Hz), 3.51 (s, 2H), 3.92–3.98 (m, 2H), 5.12 (s, 1H), 6.77–6.84 (m, 2H), 12.20 (s, 1H). MS (ESI): 215.1.

*6.1.4.6. 2-(3-Ethoxy-2-fluoro-4-hydroxyphenyl) acetic acid (**6f**).* Yield: 89%; mp: 88–90 °C. ^{1}H NMR (DMSO-d_6) δ 1.29 (t, 3H, *J* = 7.00 Hz), 3.52 (s, 2H), 3.92–3.98 (m, 2H), 5.09 (s, 1H), 6.51–6.68 (m, 2H), 12.18 (s, 1H). MS (ESI): 215.1.

*6.1.4.7. 2-(2-Fluoro-4-hydroxy-5-propoxyphenyl) acetic acid (**6g**).* Yield: 89%; mp: 86–88 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, 3H, *J* = 7.01 Hz), 1.73–1.79 (m, 2H), 3.54 (s, 2H), 4.09 (t, 2H, *J* = 7.12 Hz), 5.23 (s, 1H), 6.78–7.34 (m, 2H), 12.28 (s, 1H). MS (ESI): 229.1.

6.1.5. Synthesis of phenylacetic ester derivatives

Concentrated sulfuric acid (5 ml) was added to the solution of the compounds 6a–g (0.1 mol) and various alcohols (100 ml). The reaction mixture was refluxed for 3 h, ethyl acetate (300 ml) was added and then washed three times with distilled water and twice with saturated sodium bicarbonate. The organic phase was dried using anhydrous magnesium sulfate and evaporated under reduced pressure to obtain the products.

*6.1.5.1. Ethyl 2-(3-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**7**).* Yellow oil; Yield: 89%; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, *J* = 7.14 Hz), 3.50 (s, 2H), 3.90 (s, 3H), 4.12–4.18 (m, 2H), 5.37 (s, 1H), 6.61–6.71 (m, 2H). MS (ESI): 229.1.

*6.1.5.2. Propyl 2-(3-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**8**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, *J* = 7.41 Hz), 1.60–1.69 (m, 2H), 3.51 (s, 2H), 3.90 (s, 3H), 4.05 (t, 2H, *J* = 6.70 Hz), 5.34 (s, 1H), 6.61–6.69 (m, 2H). MS (ESI): 243.1.

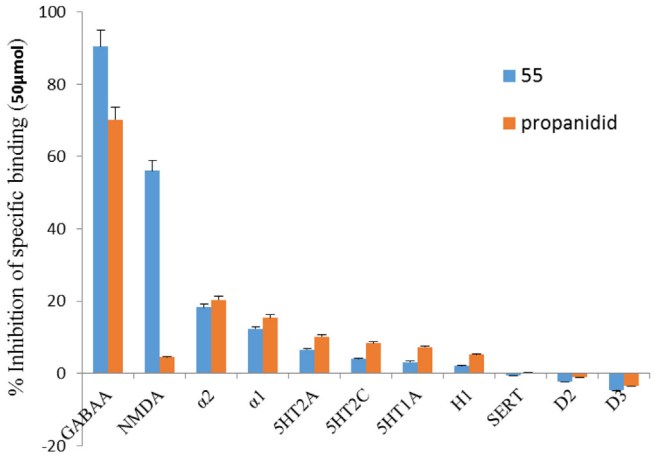


Fig. 5. Specific radioligand binding to off-target receptors by compound **55** (50 μmol). Data are expressed as means ± SD ($n = 4$).

*6.1.5.3. Isopropyl 2-(3-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**9**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 1.23 (d, 6H, $J = 6.28$ Hz), 3.47 (s, 2H), 3.90 (s, 3H), 4.98–5.10 (m, 1H), 5.71 (s, 1H), 6.61–6.71 (m, 2H). MS (ESI): 243.1.

*6.1.5.4. 2,2,2-Trifluoroethyl 2-(3-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**10**).* Yellow oil; Yield: 83%; ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 2H), 3.90 (s, 3H), 4.45–4.52 (m, 2H), 5.38 (s, 1H), 6.60–6.70 (m, 2H). MS (ESI): 283.1.

*6.1.5.5. 1,1,1-Trifluoropropan-2-yl 2-(3-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**11**).* Yellow oil; Yield: 87%; ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 3H, $J = 6.28$ Hz), 3.54 (s, 2H), 3.85 (s, 3H), 4.98–5.10 (m, 1H), 5.71 (s, 1H), 6.65–6.73 (m, 2H). MS (ESI): 297.1.

*6.1.5.6. Ethyl 2-(3-ethoxy-5-fluoro-4-hydroxyphenyl) acetate (**12**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, $J = 7.09$ Hz), 1.42 (t, 3H, $J = 7.00$ Hz), 3.56 (s, 2H), 4.05–4.18 (m, 4H), 5.73 (s, 1H), 6.66–6.72 (m, 2H). MS (ESI): 243.1.

*6.1.5.7. Propyl 2-(3-ethoxy-5-fluoro-4-hydroxyphenyl) acetate (**13**).* Yellow oil; Yield: 86%; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, $J = 7.44$ Hz), 1.41 (t, 3H, $J = 6.95$ Hz), 1.59–1.68 (m, 2H), 3.58 (s, 2H), 4.03–4.09 (m, 4H), 5.81 (s, 1H), 6.65–6.73 (m, 2H). MS (ESI): 257.1.

*6.1.5.8. Isopropyl 2-(3-ethoxy-5-fluoro-4-hydroxyphenyl) acetate (**14**).* Yellow oil; Yield: 82%; ^{1}H NMR ($CDCl_3$) δ 1.29 (d, 6H, $J = 6.32$ Hz), 1.44 (t, 3H, $J = 7.02$ Hz), 3.55 (s, 2H), 4.05–4.18 (m, 2H), 4.97–5.06 (m, 1H), 5.73 (s, 1H), 6.66–6.72 (m, 2H). MS (ESI): 257.1.

*6.1.5.9. 2,2,2-Trifluoroethyl 2-(3-ethoxy-5-fluoro-4-hydroxyphenyl) acetate (**15**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, $J = 7.02$ Hz), 3.67 (s, 2H), 4.04–4.10 (m, 2H), 4.46–4.52 (m, 2H), 5.76 (s, 1H), 6.66–6.70 (m, 2H). MS (ESI): 297.1.

*6.1.5.10. 1,1,1-Trifluoropropan-2-yl 2-(3-ethoxy-5-fluoro-4-hydroxyphenyl) acetate (**16**).* Yellow oil; Yield: 81%; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 3H, $J = 7.10$ Hz), 1.42 (t, 3H, $J = 7.00$ Hz), 3.55 (s, 2H), 4.05–4.18 (m, 4H), 5.73 (s, 1H), 6.66–6.72 (m, 2H). MS (ESI): 311.1.

*6.1.5.11. Ethyl 2-(2-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**17**).* Yellow oil; Yield: 79%; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, $J = 7.16$ Hz), 3.59 (s, 2H), 3.88 (s, 3H), 4.16–4.21 (m, 2H), 5.70 (s, 1H), 6.68–6.76 (m, 2H). MS (ESI): 229.1.

*6.1.5.12. Propyl 2-(2-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**18**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, $J = 7.32$ Hz), 1.60–1.69 (m, 2H), 3.51 (s, 2H), 3.90 (s, 3H), 4.05 (t, 2H, $J = 6.68$ Hz), 5.34 (s, 1H), 6.61–6.69 (m, 2H). MS (ESI): 243.1.

*6.1.5.13. Isopropyl 2-(2-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**19**).* Yellow oil; Yield: 86%; ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 6H, $J = 6.24$ Hz), 3.51 (s, 2H), 3.85 (s, 2H), 4.98–5.11 (m, 1H), 5.71 (s, 1H), 6.65–6.73 (m, 2H). MS (ESI): 243.1.

*6.1.5.14. 2,2,2-Trifluoroethyl 2-(2-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**20**).* Yellow oil; Yield: 81%; ^{1}H NMR ($CDCl_3$) δ 3.54 (s, 2H), 3.85 (s, 3H), 4.68–4.73 (m, 2H), 5.71 (s, 1H), 6.65–6.73 (m, 2H). MS (ESI): 283.1.

*6.1.5.15. 1,1,1-Trifluoropropan-2-yl 2-(2-fluoro-4-hydroxy-5-methoxyphenyl) acetate (**21**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 1.28 (d, 3H, $J = 7.08$ Hz), 3.55 (s, 2H), 3.90 (s, 3H), 4.95–5.01 (m, 1H), 5.73 (s, 1H), 6.66–6.72 (m, 2H). MS (ESI): 297.1.

*6.1.5.16. Ethyl 2-(5-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**22**).* Yellow oil; Yield: 87%; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, $J = 7.10$ Hz), 1.42 (t, 3H, $J = 7.00$ Hz), 3.55 (s, 2H), 4.05–4.18 (m, 4H), 5.73 (s, 1H), 6.66–6.72 (m, 2H). MS (ESI): 243.1.

*6.1.5.17. Propyl 2-(5-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**23**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, $J = 7.44$ Hz), 1.41 (t, 3H, $J = 6.95$ Hz), 1.59–1.68 (m, 2H), 3.56 (s, 2H), 4.03–4.09 (m, 4H), 5.81 (s, 1H), 6.65–6.72 (m, 2H). MS (ESI): 257.1.

*6.1.5.18. Isopropyl 2-(5-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**24**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 1.23 (d, 6H, $J = 6.28$ Hz), 1.42 (t, 3H, $J = 7.00$ Hz), 3.52 (s, 2H), 4.05–4.10 (m, 2H), 4.97–5.06 (m, 1H), 5.71 (s, 1H), 6.65–6.74 (m, 2H). MS (ESI): 257.1.

*6.1.5.19. 2,2,2-Trifluoroethyl 2-(5-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**25**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, $J = 7.02$ Hz), 3.67 (s, 2H), 4.04–4.10 (m, 2H), 4.46–4.52 (m, 2H), 5.76 (s, 1H), 6.66–6.71 (m, 2H). MS (ESI): 297.1.

*6.1.5.20. 1,1,1-Trifluoropropan-2-yl 2-(5-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**26**).* Yellow oil; Yield: 82%; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 3H, $J = 7.10$ Hz), 1.42 (t, 3H, $J = 7.00$ Hz), 3.55 (s, 2H), 4.05–4.18 (m, 2H), 4.96–5.02 (m, 1H), 5.73 (s, 1H), 6.66–6.72 (m, 2H). MS (ESI): 311.1.

*6.1.5.21. Ethyl 2-(2-fluoro-4-hydroxy-3-methoxyphenyl) acetate (**27**).* Yellow oil; Yield: 82%; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, $J = 7.14$ Hz), 3.50 (s, 2H), 3.90 (s, 3H), 4.12–4.18 (m, 2H), 5.37 (s, 1H), 6.61–6.71 (m, 2H). MS (ESI): 229.1.

*6.1.5.22. Propyl 2-(2-fluoro-4-hydroxy-3-methoxyphenyl) acetate (**28**).* Yellow oil; Yield: 83%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, $J = 7.41$ Hz), 1.60–1.69 (m, 2H), 3.51 (s, 2H), 3.90 (s, 3H), 4.05 (t, 2H, $J = 6.70$ Hz), 5.34 (s, 1H), 6.61–6.69 (m, 2H). MS (ESI): 243.1.

*6.1.5.23. Isopropyl 2-(2-fluoro-4-hydroxy-3-methoxyphenyl) acetate (**29**).* Yellow oil; Yield: 89%; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 6H, $J = 6.31$ Hz), 3.47 (s, 2H), 3.90 (s, 2H), 4.97–5.12 (m, 1H), 5.71 (s, 1H), 6.61–6.71 (m, 2H). MS (ESI): 243.1.

*6.1.5.24. 2,2,2-Trifluoroethyl 2-(2-fluoro-4-hydroxy-3-methoxyphenyl) acetate (**30**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 2H), 3.90 (s, 3H), 4.45–4.52 (m, 2H), 5.38 (s, 1H), 6.60–6.70 (m, 2H). MS (ESI): 283.1.

*6.1.5.25. 1,1,1-Trifluoropropan-2-yl 2-(2-fluoro-4-hydroxy-3-methoxyphenyl) acetate (**31**).* Yellow oil; Yield: 87%; ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 3H, J = 6.28 Hz), 3.54 (s, 2H), 3.85 (s, 3H), 4.98–5.10 (m, 1H), 5.71 (s, 1H), 6.65–6.73 (m, 2H). MS (ESI): 297.1.

*6.1.5.26. Ethyl 2-(3-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**32**).* Yellow oil; Yield: 81%; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 7.16 Hz), 1.43 (t, 3H, J = 7.00 Hz), 3.59 (s, 2H),, 4.16–4.21 (m, 4H), 5.70 (s, 1H), 6.68–6.76 (m, 2H). MS (ESI): 243.1.

*6.1.5.27. Propyl 2-(3-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**33**).* Yellow oil; Yield: 89%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 7.38 Hz), 1.43 (t, 3H, J = 7.02 Hz), 1.58–1.64 (m, 2H), 3.58 (s, 2H), 4.03–4.09 (m, 4H), 5.79 (s, 1H), 6.64–6.72 (m, 2H). MS (ESI): 257.1.

*6.1.5.28. Isopropyl 2-(3-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**34**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 6H, J = 6.28 Hz), 1.29 (t, 3H, J = 6.98 Hz), 3.54 (s, 2H), 4.05–4.18 (m, 2H), 4.98–5.10 (m, 1H), 5.71 (s, 1H), 6.65–6.73 (m, 2H). MS (ESI): 257.1.

*6.1.5.29. 2,2,2-Trifluoroethyl 2-(3-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**35**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3H, J = 6.99 Hz), 3.54 (s, 2H), 4.04–4.10 (m, 2H), 4.46–4.52 (m, 2H), 5.71 (s, 1H), 6.65–6.73 (m, 2H). MS (ESI): 297.1.

*6.1.5.30. 1,1,1-Trifluoropropan-2-yl 2-(3-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**36**).* Yellow oil; Yield: 89%; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 3H, J = 7.10 Hz), 1.42 (t, 3H, J = 7.00 Hz), 3.57 (s, 2H), 4.05–4.18 (m, 4H), 4.96–5.02 (m, 1H), 5.73 (s, 1H), 6.66–6.72 (m, 2H). MS (ESI): 311.1.

*6.1.5.31. Butyl 2-(5-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**37**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 7.44 Hz), 1.41 (t, 3H, J = 6.95 Hz), 1.59–1.68 (m, 4H), 3.56 (s, 2H), 4.03–4.09 (m, 4H), 5.81 (s, 1H), 6.65–6.72 (m, 2H). MS (ESI): 271.1.

*6.1.5.32. sec-butyl 2-(5-ethoxy-2-fluoro-4-hydroxyphenyl) acetate (**38**).* Yellow oil; Yield: 87%; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 7.23 Hz), 1.27 (t, 3H, J = 7.12 Hz), 1.42 (t, 3H, J = 7.02 Hz), 1.44–1.51 (m, 2H), 3.57 (s, 2H), 4.03–4.09 (m, 2H), 4.14–4.20 (m, 1H), 5.81 (s, 1H), 6.88–6.91 (m, 2H). MS (ESI): 271.1.

*6.1.5.33. Isopropyl 2-(2-fluoro-4-hydroxy-5-propoxyphenyl) acetate (**39**).* Yellow oil; Yield: 83%; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 6.95 Hz), 1.32 (d, 6H, J = 6.31 Hz), 1.59–1.68 (m, 2H), 3.73 (s, 2H), 4.02–4.08 (t, 2H, J = 6.68 Hz), 4.93–4.96 (m, 1H), 5.75 (s, 1H), 6.82–6.87 (m, 2H). MS (ESI): 271.1.

6.1.6. Synthesis of fluorine-substituted phenyl acetate derivatives

Compounds **7**–**39** (1 mmol), 2-chloro-N,N-diethylacetamide (1 mmol) and K_2CO_3 (1.5 mmol) were added to 50-ml acetone. The reaction mixture was refluxed overnight and the acetone was evaporated. Ethyl acetate (300 ml) was added to the residue and then washed three times with distilled water. The organic phase was dried using anhydrous magnesium sulfate and evaporated under reduced pressure. The crude product was purified using chromatography (5–10% petroleum ether/ethyl acetate) to yield the final compound.

*6.1.6.1. Ethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-fluoro-5-methoxyphenyl) acetate (**40**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.06 Hz), 1.23 (t, 3H, J = 7.06 Hz), 1.27 (t, 3H, J = 7.06 Hz), 3.39–3.50 (m, 4H), 3.53 (s, 2H), 3.87 (s, 3H), 4.14–4.20 (m, 2H), 4.69 (s, 2H), 6.64–6.69 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.77, 14.24, 40.06, 41.06, 41.35, 56.25, 61.06, 71.85, 71.87, 108.95, 109.80, 110.00, 130.05, 154.40, 156.83, 166.87, 171.04. HRMS (ESI) m/z 342.1716 ($[M+H]^+$).

*6.1.6.2. Propyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-fluoro-5-methoxyphenyl) acetate (**41**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7.47 Hz), 1.15 (t, 3H, J = 7.13 Hz), 1.23 (t, 3H, J = 7.12 Hz), 1.62–1.70 (m, 2H), 3.41–3.51 (m, 4H), 3.54 (s, 2H), 3.73 (s, 3H), 4.09 (t, 2H, J = 6.67 Hz), 4.71 (s, 2H), 6.65–6.68 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.32, 12.77, 14.24, 40.06, 41.09, 41.35, 56.25, 66.66, 71.84, 71.87, 108.92, 109.81, 110.01, 134.41, 154.39, 156.83, 166.86, 171.11. HRMS (ESI) m/z 356.1875 ($[M+H]^+$).

*6.1.6.3. Isopropyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-fluoro-5-methoxyphenyl) acetate (**42**).* Yellow oil; Yield: 86%; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.08 Hz), 1.21 (t, 3H, J = 7.08 Hz), 1.24 (d, 6H, J = 6.30 Hz), 3.38–3.48 (m, 6H), 3.49 (s, 2H), 3.73 (s, 3H), 4.67 (s, 2H), 4.96–5.06 (m, 1H), 6.62–6.67 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.78, 12.89, 21.75, 22.67, 40.95, 56.24, 62.45, 68.49, 71.86, 71.88, 108.90, 109.73, 130.35, 134.22, 153.43, 156.82, 166.89, 170.55. HRMS (ESI) m/z 356.1875 ($[M+H]^+$).

*6.1.6.4. 2,2,2-Trifluoroethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-fluoro-5-methoxyphenyl) acetate (**43**).* Yellow oil; Yield: 83%; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.10 Hz), 3.38–3.48 (m, 6H), 3.85 (s, 3H), 4.56–4.62 (m, 2H), 4.67 (s, 2H), 6.62–6.68 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.78, 14.10, 24.85, 29.27, 40.51, 56.24, 62.45, 68.49, 71.86, 108.88, 109.94, 130.26, 134.35, 153.38, 156.84, 166.94, 174.57. HRMS (ESI) m/z 396.1434 ($[M+H]^+$).

*6.1.6.5. 1,1,1-Trifluoropropan-2-yl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-fluoro-5-methoxyphenyl) acetate (**44**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.19 Hz), 1.22 (t, 3H, J = 6.97 Hz), 1.29 (d, 3H, J = 6.86 Hz), 3.39–3.44 (m, 4H), 3.59 (s, 2H), 3.85 (s, 3H), 4.71 (s, 2H), 4.98–5.04 (m, 1H), 6.71–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.25, 12.76, 14.22, 21.92, 33.96, 40.33, 41.47, 56.54, 66.56, 68.67, 102.78 (d, 1C, J = 28.13 Hz), 113.39 (d, 1C, J = 17.50 Hz), 114.23 (d, 1C, J = 5.13 Hz), 145.75, 147.60 (d, 1C, J = 9.98 Hz), 154.85 (d, 1C, J = 240.20 Hz) 166.46, 170.93. HRMS (ESI) m/z 410.1592 ($[M+H]^+$).

*6.1.6.6. Ethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-5-fluorophenyl) acetate (**45**).* Yellow oil; Yield: 89%; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.03 Hz), 1.24 (t, 3H, J = 7.02 Hz), 1.43 (t, 3H, J = 6.99 Hz), 1.43 (t, 3H, J = 7.01 Hz), 3.38–3.44 (m, 4H), 3.57 (s, 2H), 4.03–4.09 (m, 2H), 4.14–4.20 (m, 2H), 4.70 (s, 2H), 6.70–6.80 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.76, 14.15, 14.29, 14.88, 33.97, 40.29, 41.52, 60.98, 65.21, 68.74, 102.84, 113.48, 115.85, 145.01, 147.86, 154.91, 166.56, 170.93. HRMS (ESI) m/z 356.1875 ($[M+H]^+$).

*6.1.6.7. Propyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-5-fluorophenyl) acetate (**46**).* Yellow oil; Yield: 78%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 7.44 Hz), 1.15 (t, 3H, J = 7.27 Hz), 1.23 (t, 3H, J = 6.96 Hz), 1.42 (t, 3H, J = 6.96 Hz), 1.62–1.68 (m, 2H), 3.38–3.43 (m, 4H), 3.58 (s, 2H), 4.03–4.09 (m, 4H), 4.70 (s, 2H), 6.70–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.29, 12.76, 14.28, 14.88, 21.91, 33.97, 40.28, 41.52, 65.21, 66.57, 68.75, 103.10, 113.48, 115.88, 144.99, 147.86, 154.64, 166.56, 171.00. HRMS (ESI) m/z 370.2031 ($[M+H]^+$).

*6.1.6.8. Isopropyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-5-fluorophenyl) acetate (**47**).* Yellow oil; Yield: 76%; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 6.97 Hz), 1.23 (t, 3H, J = 6.97 Hz), 1.32 (d, 6H, J = 6.86 Hz), 1.45 (t, 3H, J = 7.24 Hz), 3.35–3.42 (m, 4H), 3.54 (s, 2H), 4.04–4.10 (m, 2H), 4.72 (s, 2H), 4.99–5.05 (m, 1H), 6.69–6.77 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.77, 13.84, 14.22, 21.73, 21.91, 34.25, 40.46, 56.45, 59.66, 68.41, 68.51, 102.75, 113.42, 114.04, 145.62, 147.42, 154.59, 166.45, 170.43. HRMS (ESI) m/z 370.2031 ([M+H]$^+$).

*6.1.6.9. 2,2,2-Trifluoroethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-5-fluorophenyl) acetate (**48**).* Yellow oil; Yield: 81%; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 6.97 Hz), 1.23 (t, 3H, J = 6.97 Hz), 1.44 (t, 3H, J = 7.24 Hz), 3.35–3.42 (m, 4H), 3.54 (s, 2H), 4.04–4.10 (m, 2H), 4.47–4.53 (m, 2H), 4.70 (s, 2H), 6.68–6.78 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.77, 13.84, 14.22, 21.88, 34.26, 39.95, 40.46, 56.45, 59.66, 68.46, 102.67, 113.44, 114.04, 145.61, 147.40, 154.92, 166.45, 170.43. HRMS (ESI) m/z 410.1592 ([M+H]$^+$).

*6.1.6.10. 1,1,1-Trifluoropropan-2-yl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-5-fluorophenyl) acetate (**49**).* Yellow oil; Yield: 79%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.08 Hz), 1.22 (t, 3H, J = 7.06 Hz), 1.31 (d, 3H, J = 6.87 Hz), 1.43 (t, 3H, J = 7.12 Hz), 3.38–3.43 (m, 4H), 3.69 (s, 2H), 4.03–4.08 (m, 2H), 4.71 (s, 2H), 4.99–5.05 (m, 1H), 6.70–6.78 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.75, 14.26, 14.83, 21.88, 21.91, 33.27, 39.95, 40.86, 61.19, 65.24, 68.60, 103.07, 111.95, 115.65, 123.21, 147.12, 155.69, 166.44, 169.32. HRMS (ESI) m/z 424.1702 ([M+H]$^+$).

*6.1.6.11. Ethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-5-methoxyphenyl) acetate (**50**).* Yellow oil; Yield: 82%; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.09 Hz), 1.22 (t, 3H, J = 7.09 Hz), 1.27 (t, 3H, J = 6.99 Hz), 3.38–3.43 (m, 4H), 3.54 (s, 2H), 3.83 (s, 3H), 4.15–4.21 (m, 2H), 4.71 (s, 2H), 6.71–6.80 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.77, 14.13, 14.23, 33.96, 40.33, 41.47, 56.54, 60.95, 68.66, 102.80 (d, 1C, J = 27.47 Hz), 113.34 (d, 1C, J = 17.46 Hz), 114.23 (d, 1C, J = 5.19 Hz), 145.77, 147.61 (d, 1C, J = 9.87 Hz), 154.85 (d, 1C, J = 240.38 Hz), 166.46, 170.85. HRMS (ESI) m/z 342.1716 ([M+H]$^+$).

*6.1.6.12. Propyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-5-methoxyphenyl) acetate (**51**).* Yellow oil; Yield: 81%; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7.63 Hz), 1.15 (t, 3H, J = 7.19 Hz), 1.22 (t, 3H, J = 6.97 Hz), 1.61–1.70 (m, 2H), 3.39–3.44 (m, 4H), 3.59 (s, 2H), 3.85 (s, 3H), 4.08 (t, 2H, J = 6.68 Hz), 4.71 (s, 2H), 6.71–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.25, 12.76, 14.22, 21.92, 33.96, 40.33, 41.47, 56.54, 66.56, 68.67, 102.78 (d, 1C, J = 28.13 Hz), 113.39 (d, 1C, J = 17.50 Hz), 114.23 (d, 1C, J = 5.13 Hz), 145.75, 147.60 (d, 1C, J = 9.98 Hz), 154.85 (d, 1C, J = 240.20 Hz), 166.46, 170.93. HRMS (ESI) m/z 356.1875 ([M+H]$^+$).

*6.1.6.13. Isopropyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-5-methoxyphenyl) acetate (**52**).* Yellow oil; Yield: 89%; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 6.97 Hz), 1.29 (t, 3H, J = 7.24 Hz), 1.23 (d, 6H, J = 6.24 Hz), 3.35–3.42 (m, 4H), 3.54 (s, 2H), 3.83 (s, 3H), 4.70 (s, 2H), 4.99–5.05 (m, 1H), 6.69–6.77 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.77, 13.84, 14.22, 21.91, 34.25, 40.33, 41.46, 56.45, 68.41, 68.51, 102.75, 113.35, 114.04, 145.60, 147.48, 154.59, 166.45, 170.43. HRMS (ESI) m/z 356.1875 ([M+H]$^+$).

*6.1.6.14. 2,2,2-Trifluoroethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-5-methoxyphenyl) acetate (**53**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.08 Hz), 1.23 (t, 3H, J = 7.08 Hz), 3.38–3.43 (m, 4H), 3.69 (s, 2H), 3.73 (s, 3H), 4.47–4.53 (m, 2H), 4.71 (s, 2H), 6.70–6.78 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.75, 14.26, 23.26, 33.28, 60.09, 60.83, 61.19, 65.24, 68.60, 102.80, 112.03, 115.62, 145.11, 148.23, 154.79, 166.44, 169.32. HRMS (ESI) m/z 396.1389 ([M+H]$^+$).

*6.1.6.15. 1,1,1-Trifluoropropan-2-yl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-5-methoxyphenyl) acetate (**54**).* Yellow oil; Yield: 87%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.10 Hz), 1.23 (t, 3H, J = 7.10 Hz), 1.29 (d, 3H, J = 6.96 Hz), 3.39–3.44 (m, 4H), 3.83 (s, 3H), 4.03–4.09 (m, 2H), 4.72 (s, 2H), 5.29–5.36 (m, 1H), 6.70–6.80 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.75, 13.44, 14.27, 33.61, 40.33, 41.55, 60.99, 65.22, 67.46, 68.67, 102.83, 113.10, 115.60, 145.07, 147.89, 155.68, 166.68, 169.24. HRMS (ESI) m/z 410.1592 ([M+H]$^+$).

*6.1.6.16. Ethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-5-ethoxy-2-fluorophenyl) acetate (**55**).* Yellow oil; Yield: 87%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.12 Hz), 1.22 (t, 3H, J = 7.12 Hz), 1.27 (t, 3H, J = 7.12 Hz), 1.42 (t, 3H, J = 7.02 Hz), 3.38–3.44 (m, 4H), 3.57 (s, 2H), 4.03–4.09 (m, 2H), 4.14–4.20 (m, 2H), 4.70 (s, 2H), 6.70–6.80 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.76, 14.15, 14.29, 20.88, 33.97, 40.29, 41.52, 60.98, 65.21, 68.74, 102.84, 113.31, 115.85, 144.98(d, 1C, J = 2.98 Hz), 147.92 (d, 1C, J = 10.08 Hz), 153.07 (d, 1C, J = 241.20 Hz), 166.56, 170.93. HRMS (ESI) m/z 356.1875 ([M+H]$^+$).

*6.1.6.17. Propyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-5-ethoxy-2-fluorophenyl) acetate (**56**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 7.44 Hz), 1.15 (t, 3H, J = 7.27 Hz), 1.23 (t, 3H, J = 6.96 Hz), 1.42 (t, 3H, J = 6.96 Hz), 1.62–1.68 (m, 2H), 3.38–3.43 (m, 4H), 3.58 (s, 2H), 4.03–4.09 (m, 4H), 4.70 (s, 2H), 6.70–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.29, 12.76, 14.28, 14.88, 21.91, 33.98, 40.28, 41.52, 65.21, 66.57, 68.75, 102.83, 113.36, 115.91, 145.01, 147.81, 153.68 (d, 1C, J = 243.68 Hz), 166.56, 171.00. HRMS (ESI) m/z 370.2031 ([M+H]$^+$).

*6.1.6.18. Isopropyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-5-ethoxy-2-fluorophenyl) acetate (**57**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.27 Hz), 1.22 (t, 3H, J = 7.44 Hz), 1.32 (d, 6H, J = 6.96 Hz), 1.42 (t, 3H, J = 6.96 Hz), 3.38–3.43 (m, 4H), 3.58 (s, 2H), 4.05–4.10 (m, 2H), 4.72 (s, 2H), 4.95–5.01 (m, 1H), 6.71–6.78 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.28, 12.76, 14.36, 14.88, 21.93, 33.96, 40.28, 41.52, 65.21, 66.57, 68.75, 102.83, 113.53, 115.85, 144.98, 147.91, 156.07 (d, 1C, J = 238.72 Hz), 165.67, 172.11. HRMS (ESI) m/z 370.2031 ([M+H]$^+$).

*6.1.6.19. 2,2,2-Trifluoroethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-5-ethoxy-2-fluorophenyl) acetate (**58**).* Yellow oil; Yield: 82%; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 7.02 Hz), 1.23 (t, 3H, J = 6.98 Hz), 1.42 (t, 3H, J = 7.00 Hz), 3.39–3.44 (m, 4H), 3.61 (s, 2H), 4.03–4.09 (m, 2H), 4.48–4.54 (m, 2H), 4.70 (s, 2H), 6.70–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.78, 14.28, 14.82, 21.91, 33.96, 40.28, 41.52, 65.21, 66.57, 68.75, 102.83, 113.36, 115.91, 145.65, 147.81, 155.68, 166.58, 171.09. HRMS (ESI) m/z 410.1592 ([M+H]$^+$).

*6.1.6.20. 1,1,1-Trifluoropropan-2-yl 2-(4-(2-(diethylamino)-2-oxoethoxy)-5-ethoxy-2-fluorophenyl) acetate (**59**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.27 Hz), 1.23 (t, 3H, J = 6.96 Hz), 1.43 (d, 3H, J = 6.96 Hz), 3.38–3.43 (m, 4H), 3.58 (s, 2H), 4.03–4.09 (m, 2H), 4.70 (s, 2H), 4.93–4.96 (m, 1H), 6.70–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.76, 14.26, 14.88, 21.91, 33.96, 33.98, 40.28, 41.52, 65.21, 66.57, 68.75, 103.10, 113.53, 115.85, 144.98, 147.91, 155.67, 166.56, 171.00. HRMS (ESI) m/z 424.1702 ([M+H]$^+$).

*6.1.6.21. Ethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-3-methoxyphenyl) acetate (**60**).* Yellow oil; Yield: 85%; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.06 Hz), 1.24 (t, 3H, J = 7.06 Hz), 1.28 (t, 3H, J = 7.06 Hz), 3.39–3.50 (m, 4H), 3.53 (s, 2H), 3.82 (s, 3H), 4.14–4.20 (m, 2H), 4.69 (s, 2H), 6.64–6.69 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.77,

14.15, 24.24, 40.06, 41.07, 41.35, 56.25, 61.06, 71.86, 108.95, 109.80, 110.00, 130.14, 153.46, 156.83, 166.87, 171.04. MS (ESI): HRMS (ESI) *m/z* 342.1716 ($[M+H]^+$).

*6.1.6.22. Propyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-3-methoxyphenyl) acetate (**61**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7.12 Hz), 1.15 (t, 3H, J = 7.13 Hz), 1.23 (t, 3H, J = 7.12 Hz), 1.62–1.70 (m, 2H), 3.39–3.50 (m, 4H), 3.54 (s, 2H), 3.87 (s, 3H), 4.07 (t, 2H, J = 6.67 Hz), 4.69 (s, 2H), 6.64–6.69 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.32, 12.77, 14.24, 21.91, 40.06, 41.09, 41.35, 56.25, 66.66, 71.87, 108.92, 109.81, 110.01, 134.41, 153.46, 156.83, 166.86, 171.11. HRMS (ESI) *m/z* 356.1875 ($[M+H]^+$).

*6.1.6.23. Isopropyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-3-methoxyphenyl) acetate (**62**).* Yellow oil; Yield: 86%; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.08 Hz), 1.21 (t, 3H, J = 7.08 Hz), 1.24 (d, 6H, J = 6.30 Hz), 3.38–3.48 (m, 6H), 3.85 (s, 3H), 4.47–4.53 (m, 2H), 4.67 (s, 2H), 4.96–5.06 (m, 1H), 6.62–6.67 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.78, 12.89, 14.14, 24.85, 40.51, 40.95, 41.38, 56.24, 68.49, 71.86, 108.90, 109.73, 112.24, 134.35, 154.38, 156.82, 166.89, 170.55. HRMS (ESI) *m/z* 356.1875 ($[M+H]^+$).

*6.1.6.24. 2,2,2-Trifluoroethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-3-methoxyphenyl) acetate (**63**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.08 Hz), 1.21 (t, 3H, J = 7.08 Hz), 3.38–3.44 (m, 4H), 3.55 (s, 2H), 3.82 (s, 3H), 4.47–4.53 (m, 2H), 4.68 (s, 2H), 6.61–6.68 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.78, 14.14, 24.85, 40.07, 40.51, 41.38, 56.24, 68.49, 71.86, 108.88, 109.72, 118.63, 134.22, 154.34, 155.41, 165.87, 171.55. HRMS (ESI) *m/z* 396.1389 ($[M+H]^+$).

*6.1.6.25. 1,1,1-Trifluoropropan-2-yl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-3-methoxyphenyl) acetate (**64**).* Yellow oil; Yield: 79%; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7.63 Hz), 1.15 (t, 3H, J = 7.19 Hz), 1.28 (d, 3H, J = 6.70 Hz), 1.61–1.70 (m, 2H), 3.39–3.44 (m, 4H), 3.59 (s, 2H), 3.85 (s, 3H), 4.71 (s, 2H), 4.95–5.01 (m, 1H), 6.71–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.25, 12.76, 14.22, 21.92, 33.96, 40.33, 41.47, 56.54, 66.56, 68.67, 102.78 (d, 1C, J = 27.13 Hz), 113.39 (d, 1C, J = 17.50 Hz), 114.23 (d, 1C, J = 5.26 Hz), 145.75, 147.60 (d, 1C, J = 9.88 Hz), 154.85 (d, 1C, J = 239.57 Hz), 166.46, 170.93. HRMS (ESI) *m/z* 410.1592 ($[M+H]^+$).

*6.1.6.26. Ethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-2-fluorophenyl) acetate (**65**).* Yellow oil; Yield: 82%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.09 Hz), 1.22 (t, 3H, J = 7.09 Hz), 1.45 (t, 3H, J = 7.23 Hz), 3.39–3.44 (m, 4H), 3.59 (s, 2H), 3.85 (s, 3H), 4.15–4.21 (m, 2H), 4.71 (s, 2H), 6.71–6.80 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.77, 14.13, 14.23, 33.96, 40.33, 41.47, 56.54, 60.95, 68.66, 102.80 (d, 1C, J = 27.47 Hz), 113.34 (d, 1C, J = 17.46 Hz), 114.23 (d, 1C, J = 5.19 Hz), 145.77, 147.61 (d, 1C, J = 9.87 Hz), 154.85 (d, 1C, J = 240.38 Hz) 166.46, 170.85. HRMS (ESI) *m/z* 356.1875 ($[M+H]^+$).

*6.1.6.27. Propyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-2-fluorophenyl) acetate (**66**).* Yellow oil; Yield: 84%; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7.63 Hz), 1.15 (t, 3H, J = 7.19 Hz), 1.22 (t, 3H, J = 6.97 Hz), 1.42 (t, 3H, J = 7.01 Hz), 1.61–1.70 (m, 2H), 3.39–3.44 (m, 4H), 3.59 (s, 2H), 4.02–4.10 (m, 4H), 4.71 (s, 2H), 6.71–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.25, 12.76, 14.22, 21.92, 33.96, 40.33, 41.47, 56.54, 66.56, 68.67, 102.78 (d, 1C, J = 28.13 Hz), 113.39 (d, 1C, J = 17.50 Hz), 114.23 (d, 1C, J = 5.13 Hz), 145.75, 147.60 (d, 1C, J = 9.98 Hz), 154.85 (d, 1C, J = 240.20 Hz), 166.46, 170.93. HRMS (ESI) *m/z* 370.2031 ($[M+H]^+$).

*6.1.6.28. Isopropyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-2-fluorophenyl) acetate (**67**).* Yellow oil; Yield: 86%; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 6.97 Hz), 1.23 (t, 3H, J = 7.24 Hz), 1.32 (d, 6H, J = 6.24 Hz), 1.44 (t, 3H, J = 6.98 Hz), 3.35–3.42 (m, 4H), 3.54 (s, 2H), 4.01–4.07 (m, 2H), 4.70 (s, 2H), 4.99–5.05 (m, 1H), 6.69–6.77 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.77, 13.84, 14.22, 21.73, 21.91, 34.26, 40.46, 41.46, 59.66, 68.41, 68.51, 102.66, 113.43, 114.04, 145.63, 147.42, 154.59, 166.45, 170.43. HRMS (ESI) *m/z* 370.2031 ($[M+H]^+$).

*6.1.6.29. 2,2,2-Trifluoroethyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-2-fluorophenyl) acetate (**68**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.08 Hz), 1.23 (t, 3H, J = 7.08 Hz), 1.41 (t, 3H, J = 6.88 Hz), 3.38–3.43 (m, 4H), 3.69 (s, 2H), 4.03–4.08 (m, 2H), 4.47–4.53 (m, 2H), 4.71 (s, 2H), 6.70–6.78 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.75, 14.26, 14.83, 33.27, 45.35, 60.33, 60.83, 61.19, 65.24, 68.60, 102.80, 112.03, 115.62, 121.45, 147.10, 155.69, 166.44, 169.32. HRMS (ESI) *m/z* 410.1592 ($[M+H]^+$).

*6.1.6.30. 1,1,1-Trifluoropropan-2-yl 2-(4-(2-(diethylamino)-2-oxoethoxy)-3-ethoxy-2-fluorophenyl)acetate (**69**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.10 Hz), 1.23 (t, 3H, J = 7.10 Hz), 1.29 (d, 3H, J = 7.02 Hz), 1.43 (t, 3H, J = 6.96 Hz), 3.39–3.44 (m, 4H), 3.64 (s, 2H), 4.03–4.09 (m, 2H), 4.72 (s, 2H), 5.01–5.08 (m, 1H), 6.70–6.80 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.75, 13.44, 14.27, 14.84, 33.61, 40.33, 41.55, 60.99, 65.22, 67.36, 68.67, 102.83, 113.10, 115.60, 122.6, 145.07, 156.12, 166.66, 169.24. HRMS (ESI) *m/z* 424.1702 ($[M+H]^+$).

*6.1.6.31. Butyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-5-ethoxy-2-fluorophenyl) acetate (**70**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 7.44 Hz), 1.15 (t, 3H, J = 7.27 Hz), 1.23 (t, 3H, J = 6.96 Hz), 1.43 (t, 3H, J = 6.96 Hz), 1.43–1.49 (m, 2H), 1.62–1.68 (m, 2H), 3.38–3.43 (m, 4H), 3.58 (s, 2H), 4.03–4.09 (m, 4H), 4.70 (s, 2H), 6.70–6.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 9.38, 10.29, 12.76, 14.28, 14.88, 21.91, 33.97, 40.28, 41.52, 65.21, 66.57, 68.75, 103.10, 113.53, 115.88, 128.35, 146.98, 156.07, 166.56, 171.00. HRMS (ESI) *m/z* 384.2142 ($[M+H]^+$).

*6.1.6.32. sec-Butyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-5-ethoxy-2-fluorophenyl) acetate (**71**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 7.23 Hz), 1.15 (t, 3H, J = 7.12 Hz), 1.23 (t, 3H, J = 7.12 Hz), 1.27 (d, 3H, J = 7.12 Hz), 1.42 (t, 3H, J = 7.02 Hz), 1.44–1.51 (m, 2H), 3.38–3.44 (m, 4H), 3.57 (s, 2H), 4.03–4.09 (m, 2H), 4.84–4.91 (m, 1H), 4.70 (s, 2H), 6.88–6.91 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 10.67, 12.76, 14.15, 14.29, 24.88, 33.95, 33.97, 40.29, 41.52, 60.98, 65.21, 68.74, 102.84, 113.48, 115.91, 123.69, 145.01, 155.68, 166.56, 170.93. HRMS (ESI) *m/z* 384.2142 ($[M+H]^+$).

*6.1.6.33. Isopropyl 2-(4-(2-(diethylamino)-2-oxoethoxy)-2-fluoro-5-propoxyphenyl) acetate (**72**).* Yellow oil; Yield: 88%; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.23 Hz), 1.15 (t, 3H, J = 7.12 Hz), 1.22 (t, 3H, J = 7.09 Hz), 1.29 (d, 6H, J = 6.68 Hz), 1.44–1.51 (m, 2H), 3.38–3.44 (m, 4H), 3.57 (s, 2H), 4.03–4.09 (m, 2H), 4.70 (s, 2H), 4.93–4.96 (m, 1H), 6.87–6.90 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 12.56, 12.86, 14.23, 21.28, 24.79, 33.84, 33.95, 40.31, 42.61, 62.23, 67.68, 69.96, 103.12, 113.31, 115.85, 126.88, 147.82, 156.07, 167.48, 169.86. HRMS (ESI) *m/z* 384.2142 ($[M+H]^+$).

6.2. Octanol:water partition coefficients

One milligram of each hypnotic was added to 10 ml of water buffered with 10 mm Tris (pH 7.4) and 1 ml of octanol. The mixture was stirred overnight and then centrifuged to fully separate the organic and aqueous phases. The relative hypnotic concentration in each phase (i.e., the partition coefficient) was determined by high-performance liquid chromatography as described for blood [17].

6.3. Drug formulation

10 mg/ml emulsion was prepared through the following procedure. Test Compound (1.0% w/v), soybean oil (10.0% w/v), egg phosphatide (1.2% w/v), glycerol (2.5% w/v), oleic acid (0.03% w/v) and water for injection were mixed at 60 °C, the pH was adjusted to 8 by 0.1 mol/L NaOH. The mixed solution was stirred with a Polytron tissue homogenizer for 5 min at maximum speed to provide the premixed solution. The premixed solution was circulated through the microfluidizer (12,000–15,000 psi) for 30 min to get the final emulsion.

6.4. Pharmacological methods

6.4.1. Animals

Sprague–Dawley rats (male, 250 ± 5.0 g, 8 weeks) were used. All the animals were purchased from Xuzhou Medical College (Jiangsu, China). The animals were housed under standardized conditions in terms of light and temperature, and received standard rat chow and tap water ad libitum. The animals were randomly assigned to different experimental groups, each kept in a separate cage. All animal research in this study followed the guidelines of the byelaw of experiments on animals, and was approved by the Ethics and Experimental Animal Committee of Jiangsu Nhwa Pharmaceutical Co., Ltd.

6.4.2. In vitro binding assays

6.4.2.1. $GABA_A$ binding assay. Membrane suspensions were thawed and centrifuged in incubation buffer at 10,000 × *g* for 10 min and washed by a similar centrifugation. For displacement studies, membrane suspensions (5 mg original wet wt./ml) were incubated with 0.5 nM [^{3}H]EBOB (26.2 μCi/mmol, Perkin Elmer Life Sciences, Boston, MA, USA) in the absence or presence of 50 μM of new compounds or reference drug for 2 h at 25 °C. Nonspecific binding was determined in the presence of 10 μM picrotoxin. Triplicate 1 ml samples were filtered on Whatman GF/B filters under vacuum with a Brandel Harvester and washed with 3 × 3 ml ice-cold buffer. Radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.2. NMDA binding assay. Membrane suspensions were thawed and centrifuged in incubation buffer at 10,000 × *g* for 10 min and washed by a similar centrifugation. For displacement studies, membrane suspensions (5 mg original wet wt./ml) were incubated with 2 nM [^{3}H]MK801 (22.3 μCi/mmol, Perkin Elmer Life Sciences, Boston, MA, USA) in the absence or presence of 50 μM of new compounds or reference drug for 1 h at 22 °C. Nonspecific binding was determined in the presence of 10 μM MK801. Triplicate 1 ml samples were filtered on Whatman GF/B filters under vacuum with a Brandel Harvester and washed with 3 × 3 ml ice-cold buffer. Radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.3. 5-HT_{1A} binding assay. Rat cerebral cortex was homogenized in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7) using an ULTRA TURAX homogenizer, and was then centrifuged at 32,000 *g* for 10 min. The resulting pellet was then resuspended in the same buffer, incubated for 10 min at 37 °C, and centrifuged at 32,000 *g* for 10 min. The final pellet was resuspended in Tris–HCl buffer containing 10 μM Pargyline, 4 mM $CaCl_2$ and 0.1% ascorbic acid. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 0.5 nM [^{3}H]8-OH-DPAT (187.4 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL Tris–HCl buffer containing 10 μM Pargyline, 4 mM $CaCl_2$ and 0.1% ascorbic acid. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]8-OH-DPAT, 50 μL of 10 μM serotonin. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]8-OH-DPAT, 50 μL of 50 μM new compounds. The tubes were incubated at 37 °C for 30 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.4. 5-HT_{2A} binding assay. Rat cerebral cortex was homogenized in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7) using an ULTRA TURAX homogenizer, and centrifuged at 32,000 *g* for 20 min. The resulting pellet was resuspended in the same quantity of the buffer centrifuged for 20 min. The final pellet was resuspended in 50 volumes of the Tris–HCl buffer. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 0.6 nM [^{3}H]ketanserin (60.0 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL Tris–HCl buffer. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H] ketanserin, 50 μL of 10 μM methisergide. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H] ketanserin, 150 μL of 50 μM new compounds. The tubes were incubated at 37 °C for 15 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.5. 5-HT_{2C} binding assay. Rat cerebral cortex was homogenized in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7) using ULTRA TURAX homogenizer, and centrifuged at 32,000 *g* for 20 min. The resulting pellet was resuspended in the same quantity of the buffer centrifuged for 20 min. The final pellet was resuspended in 50 volumes of the Tris–HCl buffer. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H] mesulergine, 50 μL Tris–HCl buffer. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H] mesulergine (85.4 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL of 10 μM mianserin. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]mesulergine, 50 μL of 50 μM new compounds. The tubes were incubated at 37 °C for 15 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.6. 5-HTT binding assay. Adult rat cerebral cortex was homogenized in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) using an ULTRA TURAX homogenizer, and was then centrifuged at 20,000 *g* for 10 min. The resulting pellet was then resuspended in the same buffer, incubated for 10 min at 37 °C, and centrifuged at 32,000 *g* for 10 min. The final pellet was resuspended in Tris–HCl buffer containing 150 mM NaCl and 5 mM KCl. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]paroxetine (22.9 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL Tris–HCl buffer containing 150 mM NaCl and 5 mM KCl. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]paroxetine, 50 μL of 10 μM paroxetine. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]paroxetine, 50 μL of 50 μM new compounds. The tubes were incubated at 22 °C for 60 min. The

incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.7. D_2 binding assay. Rat striatum was homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.7) using an ULTRA TURAX homogenizer, and centrifuged twice for 10 min at 48,000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris–HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 5 μM pargyline. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 0.5 nM [^{3}H]spiperone (16.2 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL Tris–HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 5 μM pargyline. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]spiperone, 50 μL of 5 μM (+)-butaclamol. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]spiperone, 50 μL of 50 μM new compounds. The tubes were incubated at 37 °C for 15 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.8. D_3 binding assay. Rat olfactory tubercle was homogenized in 20 volumes of ice-cold 50 mM Hepes Na (pH 7.5) using an ULTRA TURAX homogenizer, and centrifuged twice for 10 min at 48,000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM Hepes Na, pH 7.5, containing 1 mM EDTA, 0.005% ascorbic acid, 0.1% albumin, and 200 nM eliprodil. Total binding each assay tube was added 900 μL of membranes, 50 μL of 0.6 nM [^{3}H]7-OH-DPAT (50 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL of 50 mM Hepes Na, pH 7.5, containing 1 mM EDTA, 0.005% ascorbic acid, 0.1% albumin, 200 nM eliprodil. Non-specific binding each assay tube was added 900 μL of membranes, 50 μL of [^{3}H]7-OH-DPAT, 50 μL of 1 μM dopamine. Specific binding each assay tube was added 900 μL of Membranes, 50 μL of [^{3}H]7-OH-DPAT, 50 μL of 50 μM new compounds. The tubes were incubated at 25 °C for 60 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.9. H_1 binding assay. Guinea pig cerebellum was homogenized in 20 volumes of ice-cold 50 mM phosphate buffer (pH = 7.4) using an ULTRA TURAX homogenizer, and centrifuged twice for 10 min at 50,000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in phosphate buffer. Total binding each assay tube was added 900 μL of membranes 50 μL of 1 nM [^{3}H] mepyramine (20.0 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL phosphate buffer. Non-specific binding each assay tube was added 900 μL of membranes, 50 μL of [^{3}H]mepyramine, 50 μL of 1 μM promethazine. Specific binding each assay tube was added 900 μL of Membranes, 50 μL of [^{3}H]mepyramine, 50 μL of 50 μM new compounds. The tubes were incubated at 30 °C for 60 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.10. α_1 binding assay. Rat cerebral cortex was homogenized in 20 volumes of ice-cold Tris–HCl buffer containing 5 mM EDTA (50 mM, pH 7.7) using ULTRA TURAX homogenizer, and centrifuged at 44,000 *g* for 20 min at 4 °C. The resulting pellet was resuspended in the same quantity of the buffer centrifuged for 20 min. The final pellet was resuspended in 50 volumes of the Tris–HCl buffer. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]prazosin (85.4 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL Tris–HCl buffer. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]prazosin, 50 μL of 10 μM prazosin. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]prazosin, 50 μL of 50 μM new compounds. The tubes were incubated at 25 °C for 60 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.4.2.11. α_2 binding assay. Rat cerebral cortex was homogenized in 20 volumes of ice-cold Tris–HCl buffer containing 5 mM EDTA (50 mM, pH 7.7) using ULTRA TURAX homogenizer, and centrifuged at 44,000 *g* for 20 min at 4 °C. The resulting pellet was resuspended in the same quantity of the buffer centrifuged for 20 min. The final pellet was resuspended in 50 volumes of the Tris–HCl buffer. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]rauwolscine, 50 μL Tris–HCl buffer. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]rauwolscine (73.0 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL of 10 μM rauwolscine. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]rauwolscine, 50 μL of 50 μM new compounds. The tubes were incubated at 25 °C for 60 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 ml cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 ml) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.5. Behavioral tests

6.5.1. Hypnotic potency (HD_{50})

All tests were performed during the light period. Groups of rats were injected intravenously (lateral tail vein) over 10 s with each dose of test compound, placed in separate boxes to reduce external stimuli and assessed for the loss of righting reflex. Dosing was performed using mg/kg scheme. A set of dose levels was chosen, and depending on whether the loss of righting reflex was observed, extra doses were introduced to provide data for the loss of righting reflex over a narrow dose range to allow potency calculations. From the percentage of rats in each group showing loss of righting reflex for ≥30 s, a probit analysis (SAS Institute) was performed to obtain an HD_{50} for each compound with 95% confidence limits [18].

6.5.2. The duration of LORR

Immediately following the injection, rats were tested for loss of righting reflex. If an immediate loss did not occur, the rats were observed closely and placed on their backs to determine the time of the loss of righting reflex. Once a loss was observed, the interval between the loss of righting reflex and the return to righting reflex was recorded. The anesthetized animals recovered righting reflexes

spontaneously. To compare the duration of LORR of each compound at equipotent doses, groups of rats were injected with each compound at 2 × HD_{50} over 10 s [18].

6.5.3. Time to walk

Time to walk was the time required after righting before rats walked from the center to the periphery of a 30-cm diameter disc.

6.5.4. Time to behavioral recovery

The rotarod test was conducted to evaluate the recovery to the motor coordination. Before the LORR test, rats were trained for 2 consecutive days. Trained mice could maintain their balance on rod for more than 2 min. After the rats could walk from the center to the periphery of a 30-cm diameter disc, they were put on the rotarod. Rats were classified as behavioral recovery when, on being placed on the rod, they could maintain their balance for 20 s. The time to behavioral recovery was the time required after rats walked from the center to the periphery of a 30-cm diameter disc before they could maintain their balance on rod for 20 s.

6.5.5. Therapeutic Index (TI)

The LD_{50} was determined in rats and the TI was calculated as LD_{50}/HD_{50}.

6.5.6. Hypnosis in response to intravenous (IV) infusion [19]

Induction of hypnosis in rats were achieved using 2 × HD_{50} dose of test compound, and immediately after induction, infusion via the tail vein was commenced at HD_{50} dosage per min. the infusion rate was maintained for 20 min or 1 h or 3 h later. The depth of hypnosis was monitored by the magnitude of a withdrawal reflex to intermittent paw pinch provided by a pair of forceps.

6.5.7. Reflex depression

Reflex depression was assessed with an artery clip applied to the tail and toes after the induction of anesthesia. Combined scores for each group of 10 rats, the response being graded from 0 (no reflex response) to 4 (full reflex response) for each rat [20].

6.5.7.1. Statistics. Data are reported as means ± SD. Statistical analyses were performed using Microsoft Office Excel 2007. To estimate the potency of test and reference compounds, the ED_{50} values and their 95% confidence limits were calculated by using the program SPSS (Statistical Package for the Social Science).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.10.073.

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