Dual Inhibitors of Phosphodiesterase-4 and Serotonin Reuptake

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A new class of multitarget compounds was synthesized by linking a novel selective serotonin reuptake inhibitor (SSRI) to a PDE4 inhibitor. The new dual PDE4 inhibitor/SSRI showed antidepressant-like activity in the forced swim test in mice The SSRIs 2-{5-[3-(5-fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine (**14**) and 2-{5-[3-(5-fluoro-2-methoxy-phenyl)-propyl]-tetrahydro-furan-2-yl}-ethylamine (**15**) were both individually linked to the PDE4 inhibitor 4-(3,4-dimethoxy-phenyl)-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (**19**), via a five-carbon chain. The dual PDE4 inhibitor/SSRI 2-{5-[3-(5-fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine)-pentyl]-4,5,8,8a-tetrahydro-2H-phthalazin-1-one (**21**) showed potent and selective serotonin reuptake inhibition (IC_{50} value of 127 nM). The dual PDE4 inhibitor/SSRI **21** also inhibited PDE4D3 with a K_i value of 2.0 nM. The dual PDE4 inhibitor/SSRI was significantly more effective than the individual SSRI alone or fluoxetine in the forced swim test at standard doses. On a molar basis, the antidepressant-like effect of the dual PDE4 inhibitor/SSRI **21** showed a 129-fold increase in in vivo efficacy compared to fluoxetine.

Introduction

The neurodegeneration hypothesis of depression proposes that depression may be caused by maladaption of immune system and monoaminergic function.[1] Chronic antidepressant treatment up-regulates hippocampal neurogenesis and could thereby block or reverse cellular atrophy. Antidepressants imipramine ($C_{19}H_{24}N_2$) and amitriptyline ($C_{20}H_{23}N$) inhibit the uptake of serotonin (5-HT) and norepinephrine (NE) in vitro and in vivo. On the basis of clinical observations, inhibition of the reuptake of either of these monoamines is responsible in part for the mood-elevating action of these compounds.[2] Overall, the literature suggests that 5-HT may be involved in depression and elevation of 5-HT by reuptake inhibition could be clinically useful. Reuptake agents such as fluoxetine ($C_{17}H_{18}F_3NO$), sertraline ($C_{17}H_{17}Cl_2N$), citalopram ($C_{20}H_{21}FN_2O$), and fluvoxamine ($C_{15}H_{21}F_3N_2O_2$) are relatively selective serotonin reuptake inhibitors (SSRIs[a]) and selectively elevate 5-HT in vitro and in the brain of animals by inhibiting the human serotonin transporter (hSERT).[3,4]

SSRIs do not have marked efficacy in animal models of depression such as the forced swim test (FST) and SSRI antidepressants may require 1−4 weeks of administration to observe behavioral improvement involving longer-term adaptive changes in receptor sensitivity.[5] Animals treated repeatedly with SSRIs such as paroxetine ($C_{19}H_{20}FNO_3$) down-regulate the SERT.[6] The extent of 5-HT reuptake inhibition after repeated treatment is greater than that observed after acute drug treatment.[4] This results in progressive increase in extracellular 5-HT and stimulation of postsynaptic receptors.[7] SSRIs are extensively metabolized, and metabolites are found in the liver, lung, and brain. Metabolism of antidepressants may play a significant role in overall efficacy because generally, the secondary amines (i.e., desmethyl metabolites) are more potent inhibitors of the uptake of NE than of 5-HT.[4] However, this is not the case with fluoxetine because the main metabolite (i.e., nor-fluoxetine, $C_{16}H_{15}F_3NO$) was equally as selective a 5-HT reuptake inhibitor as its parent.[6] With acute treatment, SSRIs will increase 5-HT receptor-mediated cyclic adenosine monophosphate (cAMP) signaling.[8] Upon repeated treatment with SSRIs, this effect will be blunted (i.e., some tolerance development) because phosphodiesterases (PDEs) are up-regulated and cAMP hydrolysis is increased.[8]

Cellular cAMP concentrations are determined by synthesis (adenylyl cyclases) and hydrolysis (cAMP PDEs). PDEs comprise a diverse group of enzymes important as regulators of signal transduction[9,10] coded by distinct genes, (e.g., *PDE4A*, *PDE4B*, *PDE4C*, and *PDE4D*, referred to as "subtypes") and their selective inhibitors as "subtype-selective" inhibitors. PDE4 enzymes are particularly important in neuropsychopharmacology[9] because PDE4 hydrolyzes cAMP formed by stimulation of β adrenergic receptor-linked adenylyl cyclase in rat cerebral cortical slices.[11] Rolipram ($C_{16}H_{21}NO_3$), a selective PDE4 inhibitor, has antidepressant activity and produces memory-enhancing effects.[12] Increased cAMP increases the expression of a number of PDE4 variants in neurons. Thus, in the case of SSRIs, the adaptation of PDE4 that occurs in response to repeated treatment with SSRIs is homeostatic and in opposition to the acute effects of the drugs.

Selective inhibitors of PDE4 (i.e., rolipram) produce antidepressant-like effects in a number of preclinical tests sensitive to antidepressants. They antagonize the behavioral and physiological effects of reserpine ($C_{33}H_{40}N_2O_9$),[12] decrease the time of immobility in the FST in rats and mice,[13] and decrease response rate and increase reinforcement rate of rats under a differential-reinforcement-of-low-rate (DRL) schedule[14] and support the conclusion that PDE4 inhibitors have antidepressant activity.[9]

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[a] Abbreviations: SSRI, selective serotonin reuptake inhibitor; FST, forced-swim test; PDE, phosphodiesterase; cAMP, cyclic adenosine monophosphate; ICR mice, inbred Charles River mice; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxy indole acetic acid; DA, dopamine; NE, norepinephrine; hSERT, human serotonin transporter; hNET, human norepinephrine transporter; hDAT, human dopamine transporter; DRL, differential-reinforcement-of-low-rate; ANOVA, analysis of variance; Veh, vehicle.

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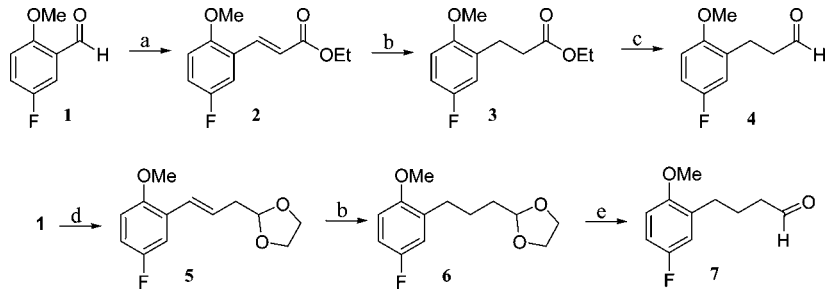
Scheme 1[a]



[a] (a) EtOC(O)CH-PPH_3, DCM; (b) H_2, Pd/C; (c) DiBAL, toluene; (d) [2-(1,3-dioxolan-2-yl)ethyl]triphenylphosphonium bromide; (e) acetone, H_2SO_4.

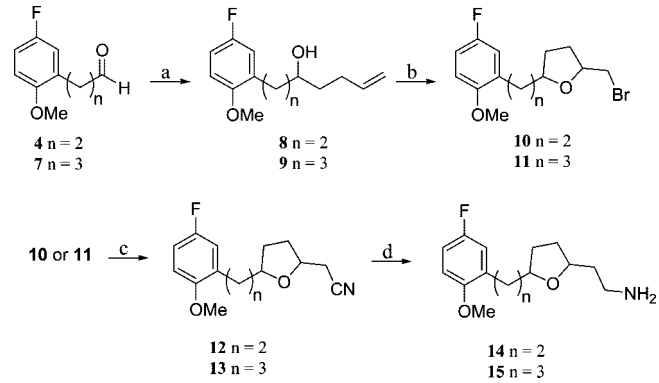
If SSRIs produce their effects in part via activation of cAMP signaling, then loss of activity of the PDE4 subtype involved would enhance their ability to increase cAMP and alter subsequent pharmacology. Thus, one adaptation of PDE4 that occurs in response to repeated treatment with SSRIs increases 5-HT receptor-mediated cAMP signaling. With repeated treatment, this effect will be blunted as tolerance develops because PDE4 is up-regulated and cAMP hydrolysis is increased. When animals are repeatedly treated with SSRIs, PDE4 is up-regulated.[7] This may be a consequence of 5-HT receptor-mediated cAMP signaling. Increased cAMP augments the expression of a number of PDE4 variants in neurons.[8]

Compared to imipramine-treated rats, a combination of rolipram and imipramine showed that chronic coadministration of a PDE4 inhibitor with an antidepressant may be more effective for depression therapy and suggest that elevation of the cAMP signal transduction pathway is involved in the antidepressive effects.[15] Likewise, acute treatment with fluoxetine to elevate synaptic 5-HT but not dopamine (DA) significantly enhanced rolipram binding and accumulation.[16] Dual PDE4 inhibitor/SSRIs offer advantages beyond simple additive effects of individual agents. One hypothesis is that dual PDE4 inhibitors/SSRIs block the effect of the up-regulation of PDE4 so that the overall increase in 5-HT-mediated cAMP signaling is preserved with repeated treatment. Herein, we describe an SSRI that was chemically linked to a PDE4 inhibitor. The dual PDE4 inhibitor/SSRI was examined in vitro and in vivo for pharmacological activity.

Results

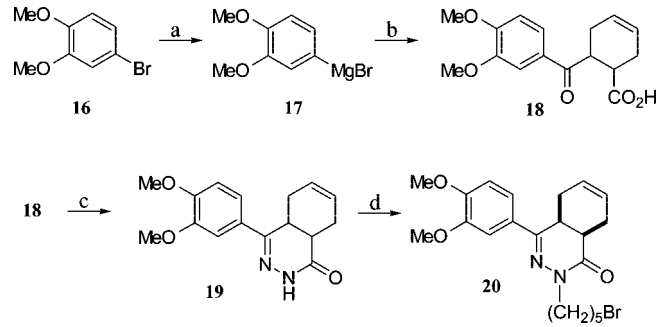
Chemistry. The chemical synthesis of the dual inhibitors consisted of coupling an SSRI (i.e., a 2,5-disubstituted tetrahydrofuran) with a known PDE4 inhibitor (i.e., a phthalazinone) via a five-carbon linker to afford the target compound. The SSRI portion of the dual inhibitor was obtained as follows. The requisite aldehyde (i.e., **4** (3-(5′-fluoro-2′-methoxyphenyl)propanal) and **7** (4-(5′-fluoro-2′-methoxyphenyl)butyraldehyde), synthesized by standard chemistry (as described in Scheme 1), was combined with butenyl magnesium bromide to afford the alcohols 7-(5′-fluoro-2′-methoxyphenyl)hept-1-en-5-ol (**8**) and 8-(5′-fluoro-2′-methoxyphenyl)oct-1-en-5-ol (**9**), respectively (Scheme 2). Cyclization of **8** and **9** in the presence of NBS afforded the 2,5-disubstituted tetrahydrofurans 2-(bromomethyl)-5-(2′-methoxy-5′-fluorophenethyl)tetrahydrafuran (**10**) and 2-(bromomethyl)-5-(3′-(2″-methoxy-5″-fluorophenyl)-1′-propyl)tetrahydrofuran (**11**), respectively (Scheme 2). Treatment of **10** or **11** with KCN resulted in the cyano compounds 2-cyanomethyl-5-(5′-fluoro-2-methoxyphenethyl)tetrahydrofuran (**12**) and 2-cyanomethyl-5-(3′-(2″-methoxy-5″-fluorophenyl)-1′-propyl)tetrahydrafuran (**13**), respectively, that were hydrogenated with H_2 in the presence of Raney nickel and ammonia to afford 2-{5-[3-(5-fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine (**14**) and 2-{5-[3-(5-fluoro-2-methoxy-phenyl)-propyl]-tetrahydro-furan-2-yl}-ethylamine (**15**), respectively. The SSRIs **14** and **15** were used to couple with the PDE4 inhibitor portion of the molecule to afford the dual PDE4/SSRI.

Scheme 2[a]



[a] (a) 1-Butenylmagnesium bromide; (b) NBS, DCM; (c) KCN, NaI, DMSO; (d) Raney Ni, H_2, 1 M NH_3 in MeOH.

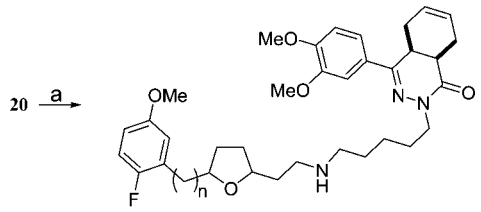
Scheme 3[a]



[a] (a) Mg turlings, THF, reflux; (b) *cis*-1,2,3,6-tetrahydrophthalic anhydride, THF; (c) H_2NNH_2, EtOH, reflux; (d) 1,5-dibromopentane, NaH, DMF.

The PDE4 inhibitor portion of the dual inhibitor was synthesized by a previously described route[17,18] (Scheme 3). Because it was known that the PDE4 inhibitor 4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (**19**) possessed increased inhibitory potency upon *N*-alkylation, we hypothesized that a more potent PDE4 inhibitor could arise from linking the SSRI and PDE4 compounds via *N*-alkylation. The final target compounds 2-{5-[3-(5-fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine)-pentyl]-4,5,8,8a-tetrahydro-2H-phthalazin-1-one (**21**) and 2-{5-[3-(5-fluoro-2-methoxy-phenyl)-propyl]-tetrahydro-furan-2-yl}-ethylamine)-pentyl-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (**22**) were prepared in a two step sequence involving: (1) attachment of a five-carbon linker by treating the phthalazinone **19** with NaH and 1,5-dibromopentane

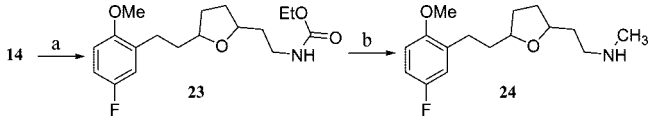
Scheme 4[a]



21 n = 2
22 n = 3

[a] (a) CsOH·H_2O, 4 Å sieves, **14** or **15**, DMF.

Scheme 5[a]



[a] (a) Ethylchloroformate, K_2CO_3, THF; (b) LAH, THF.

Table 1. Inhibition of Recombinant PDE4 Isoforms with PDE4 Inhibitors and Dual PDE4 Inhibitor/SSRIs

	enzyme		
compd	human PDE4D3 K_i (nM)	human PDE4B3 K_i (nM)	human PDE4A1 K_i (nM)
19	6.3	250	>1000
20	2.3	ND[a]	ND
21	2.0	199	>1000
22	1.2	500	>1000
Rolipram	58.9	ND	ND

[a] ND, not done.

to afford 2-(5-bromo-pentyl)-4-(3,4-dimethoxy-phenyl-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (**20**) and (2) linking **20** to **14** and **15** with the aid of CsOH (to diminish formation of the tertiary amine) to afford **21** and **22**, respectively (Scheme 4). The *N*-methyl derivative of **14** was synthesized in a two-step sequence starting with protection with ethylchloroformate in the presence of base to from **23** and then reduction of **23** to **24** with lithium aluminum hydride in the presence of THF (Scheme 5).

Biology. Inhibition of PDE4 enzymes was conducted with the synthetic agents (Table 1). In addition, binding affinity of each compound (i.e., K_i value) was calculated from the IC_{50} value and measured for each compound by assessing the potency of inhibition of binding radiolabeled **25** (RTI-55)[19] to the hDAT, hSERT, and hNET[20,21] (Table 2). In vitro functional potency was measured by determining the reuptake inhibition (i.e., IC_{50} value) of [^{3}H]-DA, [^{3}H]-5-HT, or [^{3}H]-NE at the recombinant hDAT, hSERT, or hNET in the presence of HEK-293 cells transfected with the respective transporter cDNA.

PDE4 Inhibitors. Compound **19** and rolipram are known PDE4 inhibitors, and we confirmed this observation (Table 1). It was known that *N*-substitution was beneficial for PDE4 inhibition potency,[17] and in the phthalazinone series, the *cis*-(±)-4a,5,8,8a-tetra- and *cis*-(±)-4a,5,6,7,8,8a-hexahydro-2*H*-phthalazin-1-ones showed potent inhibition of PDE4 by occupying a region of space different from that of other fused ring systems to afford selective inhibition.[17,18] *N*-Substituted derivatives distal to the fused ring system were synthesized to examine whether additional pharmacological properties could be introduced into **19** while still preserving PDE4 inhibitory activity. In fact, the *N*-substituted phthalazinone **20** was a more potent inhibitor of PDE4D3 than was **19** (Table 1). Thus, a template with potent PDE4 inhibitory activity was chemically combined with a molecule possessing potent SSRI function to make a compound with a dual PDE4 inhibition/SSRI reuptake inhibition profile.

The pattern of effects of PDE4 inhibitors on behavior is distinct from that of selective inhibitors of other PDE families.[9,14] Generalized increases in cAMP do not adequately explain the behavioral effects of PDE4 inhibitors. Thus, changes in cAMP signaling in particular pathways (e.g., β adrenergic or NMDA receptor-mediated signaling) result in the pattern of behavioral changes observed.[11] There are four PDE4 genes (i.e., *PDE4A*, *PDE4B*, *PDE4C*, and *PDE4D*). The enzyme PDE4C is mainly in the periphery and was not studied herein[9,10] but PDE4A, PDE4B, and PDE4D are present in the brain and their distribution suggest distinct roles in neuropsychopharmacology. Repeated treatment of animals with the antidepressant desipramine ($C_{18}H_{21}N_2$), which indirectly stimulates β receptors alters PDE4A and PDE4B expression.[8] The PDE4 inhibitor rolipram does not produce antidepressant-like effects in PDE4D knockout mice and PDE4D may play a role in pharmacotherapy of depression.[9,10] Consequently, the inhibition of these PDE4 forms was studied (Table 1).

Selective Serotonin Reuptake Inhibitors. Compounds **14** and **15** were derived from molecular dissection of aryltropanes[21] and were potent inhibitors of serotonin reuptake (Table 2). Compounds **14** and **15** were potent inhibitors of radiolabeled **25** binding to the hSERT (i.e., K_i values of 2.1 and 1.1 nM, respectively) and reuptake (i.e., K_i values of 2.5 and 2.3 nM, respectively) (Table 2). Compounds **14** and **15** were equal to or more potent and selective than fluoxetine in reuptake inhibition of the hSERT. For **14** and **15**, the selectivity of either binding potency or reuptake efficacy for hSERT was very great. Thus, the binding selectivity ratios K_i(hDAT)/K_i(hSERT) and K_i(hNET)/K_i(hSERT) for **14** and **15** were 3095 and 1162 and 6642 and 9636, respectively. The reuptake inhibition selectivity ratios IC_{50}(hDAT)/IC_{50}(hSERT) and IC_{50}(hNET)/IC_{50}(hSERT) for **14** and **15** were 11140 and 192 and 7386 and 839, respectively. Compounds related to the PDE4 inhibitor (i.e., **19** and **20**) were ineffective at inhibiting **25** binding to the hDAT, hSERT, or hNET (i.e., K_i values 39−100 μM).

Dual PDE4 Inhibitor/SSRIs. Compounds **19**, **21**, and **22** were tested for inhibition of cAMP hydrolysis by recombinant forms of human PDE4D3, human PDE4B3, and human PDE4A1, in vitro. The K_i value for inhibition of human PDE4D3 for rolipram, **19**, **21**, and **22** was 58.9, 6.3, 2.0, and 1.2 nM, respectively (Table 1). The increase in potency for **21** and **22** compared with **19** suggested that *N*-substitution increased inhibition of human PDE4D.[17] For human PDE4B3, the K_i values for inhibition of cAMP hydrolysis of **19**, **21**, and **22** were 250, 199, and 500 nM, respectively. In the case of human PDE4A1, compounds **19**, **21**, and **22** all showed K_i values for inhibition of cAMP hydrolysis with values greater than 1 μM. Thus, **19**, **21**, and **22** possessed considerable inhibitory selectivity for PDE4D.

The dual PDE4 inhibitor/SSRIs (i.e., compounds **21** and **22**) were moderately potent inhibitors of binding of radiolabeled **25** to the hSERT having K_i values of 156 and 194 nM, respectively. For compounds **21** and **22**, inhibition of neurotransmitter reuptake was highly selective for the hSERT (i.e., IC_{50} values of 127 and 194 nM, respectively) (Table 2). For **21** and **22**, the selectivity of either binding potency or reuptake efficacy was greatest for hSERT. Finally, the data showed that dual PDE4 inhibitor/SSRIs **21** and **22** were relatively selective for the hSERT and did not potently interact with hDAT or hNET (Table 2).

Table 2. Inhibition of Radioligand Binding and [^{3}H] Neurotransmitter Uptake in HEK-hDAT, HEK-hSERT, and HEK-hNET Cells[a]

		binding (K_i, nM)			reuptake (IC_{50}, nM)		
compd	description	hDAT	hSERT	hNET	hDAT	hSERT	hNET
cocaine		371 ± 81	276 ± 87	1115 ± 198	303 ± 74	416 ± 135	835 ± 229
Fluoxetine[b]		6670 ± 850	1.1 ± 0.5	1560 ± 300	19500 ± 7600	7.3 ± 2.9	1020 ± 180
14	SSRI	6500 ± 828	2.1 ± 0.2	2440 ± 215	27850 ± 5129	2.5 ± 0.6	481 ± 158
15	SSRI	7307 ± 1331	1.1 ± 0.1	10600 ± 1880	16987 ± 1352	2.3 ± 0.6	1930.0 ± 463.0
19	PDE4 inhibitor	>100000	>100000	>100000	>100000	>100000	>100000
20	N-modified PDE4 inhibitor	>100000	>100000	39119 ± 4915	>100000	>100000	>100000
21	dual inhibitor	2478 ± 451	156 ± 30	1748 ± 257	3556 ± 377	127 ± 15	957 ± 146
22	dual inhibitor	5340 ± 1083	194 ± 87	7441 ± 1269	5340 ± 1083	194 ± 87	7441 ± 1269
24	SSRI	66988 ± 14450	21.2 ± 4.3	19332 ± 3577	>100000	18.4 ± 6.1	20370 ± 3751

[a] Values are the mean ± SEM of three to four independent experiments conducted with duplicate (binding) or with triplicate (uptake) determinations unless the mean of three experiments exceeded 100 μM. Drug inhibition of [^{125}I]-**25** binding in HEK-hDAT, HEK-hSERT, or HEK-hNET cell membranes. Inhibition of [^{3}H]-DA, [^{3}H]-5-HT, [^{3}H]-NE in the presence of HEK-hDAT, HEK-hSERT, or HEK-hNET cells, respectively; [b] Data taken from ref 20.

In Vivo Studies. The PDE4 inhibitor (i.e., **19**), the SSRI (i.e., **14**), and the dual PDE4 inhibitor/SSRI (i.e., **21**) were separately examined for acute and subchronic antidepressant-like effects in vivo. The two compounds were not administered in combination. Acute treatment of inbred Charles River (ICR) mice with either **19** or **21**, at doses of 0.3 and 3 mg/kg, decreased duration of immobility in a dose-dependent manner in the forced-swim test (FST)[22,23] (Figure 1). Compared to vehicle control, the higher dose of **19** or **21** (3 mg/kg) significantly decreased immobility in the FST ($P < 0.05$). In contrast, acute administration of fluoxetine (40 mg/kg) or repeated treatment with rolipram (0.5 mg/kg, ip, once a day for 8 days) (data not shown), was required to produce similar antidepressant-like effects in the FST. On a molar basis, compound **21** (0.5 μmol/kg) was 129-fold more efficacious than fluoxetine (65 μmol/kg) in the FST to give the same effect. Acute treatment with rolipram (0.5 mg/kg, data not shown) did not alter FST behavior in agreement with the literature.[8] The SSRI, compound **14**, at the doses used (i.e., 10 and 30 mg/kg) tended to decrease immobility, but this effect was not statistically significant (Figure 1).

Because Balb/c mice have been reported to exhibit a relatively high immobility baseline and are sensitive to fluoxetine challenge in the FST,[23] the in vivo studies described above were repeated. Acute administration of fluoxetine (20 mg/kg), **14** (10 mg/kg), or **15** (10 mg/kg) did not markedly alter the duration of immobility in the FST in Balb/c mice (Figure 2). In contrast, subchronic administration of a lower dose of fluoxetine (10 mg/kg) significantly decreased immobility ($P < 0.05$). However, subchronic treatment with **14** (10 mg/kg) or **21** (1 mg/kg) did not change immobility compared to vehicle control (Figure 3). It is possible that the lack of in vivo activity for **14** or **15** was a consequence of metabolic instability. To test this possibility, the in vitro metabolic stability of **14**, **15**, **19**, **21**, **22**, and **24** was examined.

In Vitro Metabolic Studies. The in vitro metabolism of compounds **14** and 2-{5-[3-(5-fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine)-methyl-amine (**24**) were examined in the presence of mouse and human liver microsomes supplemented with NADPH to examine the metabolic stability of the compounds and because metabolism plays a large role in the pharmacological activity of SSRIs. We hypothesized that by blocking a prominent route of metabolism of SSRIs (i.e., aliphatic amine metabolism), a more efficacious agent could be obtained. Compounds **19**, **21**, and **22** were examined in the presence of mouse and human liver microsomes supplemented with NADPH. An efficient HPLC method was established to afford separation of the starting material from any putative metabolites (e.g., aldehyde, hydroxylamine, nitrone). Aerobic incubations of **14** and **24** in the presence of mouse and rat liver microsomes supplemented with NADPH showed a time-dependent loss of substrate. For **14** and **24**, the calculated half-life was 56 or 106 and 58 or 129 min, respectively, in the presence of mouse and rat liver microsomes (Table 3). In the presence of mouse liver microsomes, compounds **19** and **21** showed considerable metabolic stability, having calculated half-lives of 211 and 154 min, respectively. Compound **21** was not

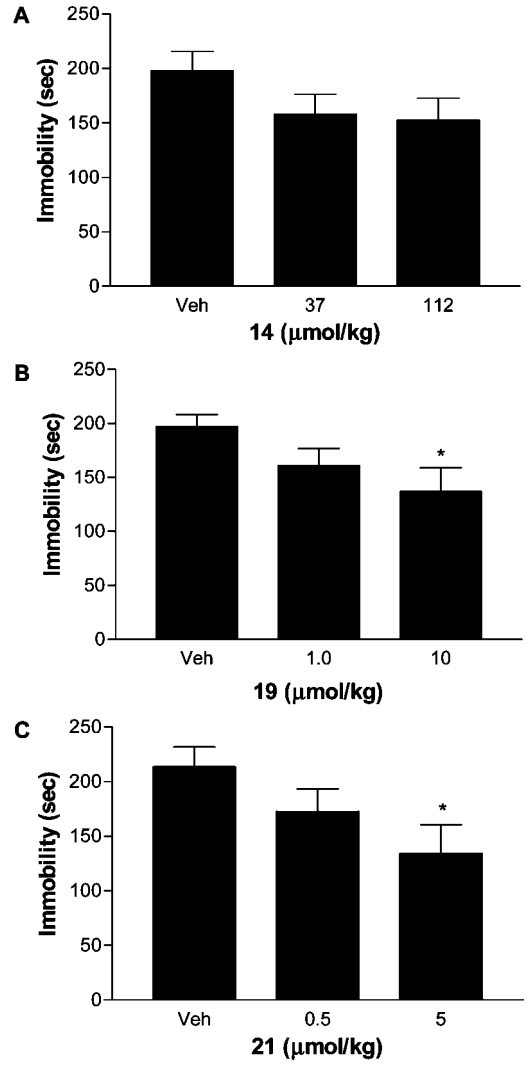


Figure 1. Effect of acute treatment of test compounds on immobility in the forced-swim test in ICR mice: (A) compound **14** (10 mg/kg, 37 μmol/kg and 30 mg/kg, 112 μmol/kg), (B) compound **19** (0.3 mg/kg, 1.0 μmol/kg and 3 mg/kg, 10 μmol/kg), and (C) compound **21** (0.3 mg/kg, 0.5 μmol/kg and 3 mg/kg, 5 μmol/kg). Vehicle (Veh) or test agent was injected ip 30 min prior to the test. $n = 9-11$; * $P < 0.05$ vs Veh.

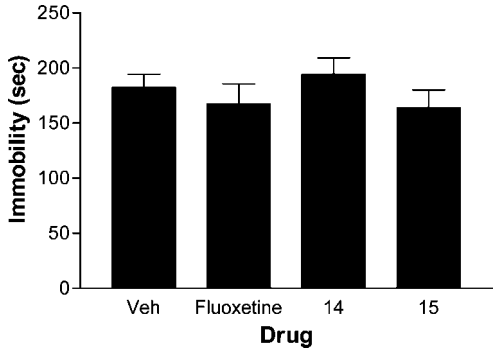


Figure 2. Effect of acute treatment with fluoxetine (20 mg/kg, 65 μmol/kg), compound **14** (10 mg/kg, 37.4 μmol/kg), and compound **15** (10 mg/kg, 35.5 μmol/kg) on immobility in the forced-swim test in Balb/c mice. Vehicle or test agent was injected ip 30 min prior to the test. $n = 10-11$.

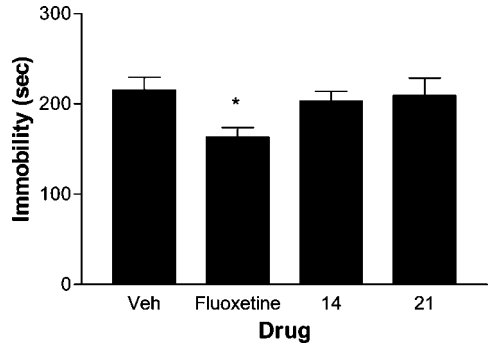


Figure 3. Effect of subchronic administration of fluoxetine (10 mg/kg, 32.3 μmol/kg), compound **14** (10 mg/kg, 37.4 μmol/kg), and compound **21** (1.0 mg/kg, 1.6 μmol/kg) on immobility in the forced-swim test in Balb/c mice. Vehicle or the test agent was injected ip 23, 5, and 1 h prior to the test. $n = 10-11$; * $P < 0.05$ vs vehicle.

Table 3. Effect of Hepatic Microsomes on the Metabolic Stability of SSRIs, PDE4 Inhibitors, and Dual PDE4 inhibitor/SSRIs

compd	RLM[a] $t_{1/2}$ (min)	MLM[b] $t_{1/2}$ (min)	HLM[c] $t_{1/2}$ (min)
14	106.3	56.0	NA[d]
19	NA	211.3	ND[e]
21	NA	153.9	ND
22	NA	ND	ND
24	129.0	58.5	NA

[a] RLM, rat liver microsomes. [b] MLM, mouse liver microsomes. [c] HLM, human liver microsomes. [d] NA, not available. [e] ND, no detectable degradation observed in the presence of animal liver microsomes supplemented with NADPH for 60 min.

detectably metabolized in the presence of human liver microsomes. No detectable metabolic instability of compound **22** was observed in the presence of either mouse or human liver microsomes up to 60 min (Table 3), and we judged these compounds were metabolically stable enough to undertake in vivo studies. Lack of efficacy of **14** and **15** was likely not due to metabolism.

Discussion

A dual PDE4 inhibitor/SSRI was synthesized by linking an SSRI with a PDE4 inhibitor via a five-carbon linker. Previously, it was shown that the potency of PDE4 inhibition of **19** was not diminished by addition of lipophilic bulk to the phthalazin-1-one nitrogen atom and **20** was a more potent PDE4D3 inhibitor than **19**. Thus, elaboration of **21** and **22** afforded PDE4 inhibitors linked to an SSRI that retained considerable pharmacological activity. Compared to **19**, compounds **21** and **22** possessed more potency at inhibiting PDE4. The dual PDE4 inhibitor/SSRIs **21** and **22** possessed considerable potency for inhibition of binding of **25** and inhibition of 5-HT reuptake in the presence of the hSERT. Compared to **14** and **15**, however, some functional activity was lost. Because studies have shown that the *trans* diastereomer of **14** or **15** possess about 7-fold more hSERT reuptake inhibition activity than the *cis* diastereomer (unpublished data) preparation of a dual inhibitor with only the *trans* diastereomer should increase potency. While some loss of in vitro binding potency and reuptake inhibition of the hSERT was observed in chemically linking SSRIs **14** and **15** to compound **20**, nevertheless, significant in vitro activity was retained.

A dual PDE4 inhibitor/SSRI offers advantages beyond simple additive effects of coadministration of the individual agents including providing greater symptomatic efficacy and better utility. The "message-address" concept of a dual agent could afford proximal inhibition of PDE4, thus keeping ample cAMP concentrations present near the activated transporter for greater functional selectivity. Decrease in the catabolism of cAMP might be expected to increase the sensitivity to and effectiveness of SSRI antidepressants. Evidence points to the PDE4D subtype as an important component of the signaling pathway involved in mediation of antidepressant effects on behavior. Dual PDE4 inhibitor/SSRIs can affect local control of 5-HT and cAMP in a stimulus-selective manner because of the compartmentalization of the PDE enzymes. In fact, acute treatment of animals with fluoxetine that elevated synaptic 5-HT and NE but not DA levels significantly enhanced rolipram binding and retention.[16] By chemically combining a selective PDE4 inhibitor with an SSRI, synergistic antidepressant effects might be observed.

The greater PDE4 inhibitor potency of the dual agent will allow lower doses to be used and decrease side effects. Because CNS disorders are recognized as polyetiological in nature, drugs that modulate multiple targets will contribute to the multifactorial processes in disease treatment. PDE4D inhibition results in a pattern of behavior that is indicative of an antidepressant-like effects.[13,14] This suggests that PDE4D is critical in mediating the antidepressant-like effects of PDE4 inhibitors. The PDE4 inhibitor **19** and the dual PDE4 inhibitor/SSRI **21** at doses of 0.3 and 3 mg/kg, respectively, decreased duration of immobility in the FST in a dose-dependent manner in ICR mice. Compared to vehicle control, 3 mg/kg significantly decreased immobility in the FST ($P < 0.05$). Acute administration of **14** decreased immobility in the FST, but this did not reach statistical significance. In contrast, 40 mg/kg of fluoxetine or repeated treatment with rolipram (0.5 mg/kg, ip, once a day for 8 days) was required to produce similar antidepressant-like effects in the FST; acute treatment with 0.5 mg/kg rolipram did not alter FST behavior.

Acute administration of fluoxetine (20 mg/kg) or **14** (10 mg/kg) did not alter the duration of immobility in the FST in Balb/c mice, but SSRIs are known to not significantly affect changes in FST after acute administration. In contrast, subchronic administration of a lower dose of fluoxetine (10 mg/kg) significantly decreased immobility ($P < 0.05$). However, subchronic treatment with **14** or **15** (10 or 1 mg/kg, respectively) did not change immobility compared to the vehicle control.

In summary, we hypothesize that dual PDE4 inhibitor/SSRIs offer an advantage over simple additive effects of coadministered agents. Dual PDE4 inhibitor/SSRIs offer the advantage of blocking the effect of the up-regulation of PDE4 in a compartmentalized manner. While PDE4 expression will still increase, its hydrolytic activity will be blocked. Thus, the overall

increase in serotonin receptor-mediated cAMP signaling will be preserved with repeated treatment. Moreover, we suggest that compounds such as **21** show the utility of addressing multiple targets to alleviate complex disease such as depression.

Experimental Section

General. Chemicals used in this study were of the highest purity available. Commercially available reagents including 2-methoxy-5-fluoro benzaldehyde were purchased from Aldrich Chemical Co. (Milwaukee, WI) or VWR (San Diego, CA) and were used as received. All moisture sensitive reactions were carried out in flame-dried glassware under an argon atmosphere. Tetrahydrofuran (THF) and toluene were freshly distilled from calcium hydride under an argon atmosphere. Methanol (CH_3OH) was passed through a column of neutral alumina and stored over 3 Å molecular sieves prior to use. Rolipram, fluoxetine, diethyleneaminetetracetic acid (DETAPAC), and all the compounds of the NADPH-generating system were obtained from Sigma Chemical Company (St. Louis, MO). Human liver, pooled female liver, and pooled male rat liver microsomes were purchased from BD Gentest Corp. (Woburn, MA). The human liver microsomes had the following functional activities (nmol/min/mg of protein): phenacetin *O*-deethylase (0.18), coumarin 7-hydroxylase (2.0), (*S*)-mephenytoin *N*-demethylase (0.05), diclofenac 4′-hydroxylase (1.9), (*S*)-mephenytoin 4′-hydroxylase (0.03), bufuralol 1′-hydroxylase (0.12), chlorzoxazone 6-hydroxylase (1.5), testosterone 6β-hydroxylase (4.6), and methyl *p*-tolyl sulfide oxidase (4.3). The pooled male rat liver microsomes had the following functional activities (nmol/min/mg of protein): testosterone 6β-hydroxylase (5.8) and nicotine oxidase (2.2). The pooled female mouse liver microsomes had the following functional activities (nmol/min/mg of protein): 7-ethoxyresorufin *O*-deethylase (0.39), *p*-nitrophenol hydroxylase (1.9), lauric acid-hydroxylase (0.86), and testosterone 6β-hydroxylase (5.0). Cocaine was provided by the National Institute on Drug Abuse, NIH (Bethesda, MD). Compound **25** was a kind gift of Dr. Ivy Carroll (RTI, Research Triangle Park, NC). [^{3}H]-DA, [^{3}H]-5-HT, [^{3}H]-NE, and [^{125}I]-**25** were purchased from Perkin-Elmer Life Sciences (Boston, MA). The preparation of the hDAT used was described previously.[20] The hSERT cDNA and HEK cells transfected with hNET cDNA was generously supplied by Dr. Randy Blakely (Vanderbilt University, Nashville, TN). Analytical thin-layer chromatography (TLC) was done on K6F silica gel 60 Å glass-backed plates from Whatman (Clifton, NJ). Compounds were detected using UV absorption at 254 nm and/or stained with I_2 (iodine). Flash chromatography was done on (60 Å) pore silica gel from E. Merck (Darmstadt, Germany).

Instrument Analysis. NMR spectra were recorded with a Bruker spectrometer operating at 500 MHz (NuMega Resonance Laboratories, Inc., San Diego, CA) or at 300 MHz by an in-house Varian spectrometer (Palo Alto, CA) using the solvent specified. Chemical shifts were reported in parts per million (ppm, δ) using residual solvent signals as internal standards. Low resolution mass spectroscopy (LRMS) was done with an HP 1100 mass spectrometer (HT Laboratories, San Diego, CA) using electrospray ionization (ESI) or with an in-house Hitachi M-8000 3DQMS (ion trap) mass spectrometer using ESI. High resolution mass spectroscopy (HRMS) was done with a Micromass LCT time-of-flight mass spectrometer at the University of Montana Mass Spectral Facility (Missoula, MT) using ESI.

The 2,5-disubstituted tetrahydrofurans and the dual PDE4 inhibitor/SSRIs were characterized by ^{1}H NMR, LRMS, and HRMS and their purities (>95%) were quantified by HPLC in two distinct solvent systems. Analytical HPLC measurements were run on a Hitachi L-6200 system equipped with a Hitachi L-7400 UV detector. Separations were done with a 5 μm, 4.6 mm × 250 mm, Axxi-chrom silica column (Richard Scientific, Novato, CA) or with a 5 μm, 4.6 mm × 250 mm Supelco HS F5 pentafluorophenyl column (Supelco Inc., Bellefonte, PA). HPLC analysis is described in the Supporting Information. ^{1}H NMR and mass spectra were consistent with the assigned structures.

Transporter Binding Assays. HEK-hDAT, -hSERT, or -hNET cells were grown until confluent as described previously.[20,24] Cells were scraped from plates and centrifuged for 20 min at 30000*g* and the pellet was resuspended in 0.32 M sucrose with a Polytron at a setting of 1 for 5 s. Assays contained 50 μL of membrane preparation, 25 μL of the test compound and 25 μL of [^{125}I]-**25** (40−80 pM final concentration) in a final volume of 250 μL Krebs HEPES buffer (25 mM HEPES, 122 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 μM pargyline, 100 μM tropolone, 2 mg glucose/mL, and 0.2 mg ascorbic acid/mL at pH 7.4.). Membranes were preincubated with test compounds for 10 min before addition of [^{125}I]-**25**. Specific binding was determined as the difference in binding observed in the presence and absence of 5 μM mazindol (HEK-hDAT and hNET) or 5 μM imipramine (HEK-hSERT). The incubations were done in the dark and terminated by filtration onto a Whatman GF/C filters using a 96-well Tomtech cell harvester (Tomtech, Orange, CT). Scintillation fluid was added to each filter spot and radioactivity remaining on the filter was determined using a Wallace β-plate reader (Wallace Laboratories, Cranbury, NJ). Specific binding was defined as the difference in binding in the presence and absence of 5 μM mazindol (hDAT and hNET) or 5 μM imipramine (hSERT).

Inhibition of Substrate Uptake. HEK-hDAT, -hSERT, or -hNET cells were grown as described above. Cells were scraped from the plates and suspended cells were added to a 96-well plate containing test compounds and Krebs-HEPES buffer in a final assay volume of 0.5 mL. After a 10 min preincubation in a 25 °C water bath, [^{3}H]-labeled neurotransmitter (50 μL, 20 nM final concentration) was added and the assay was initiated. After 10 min, the incubation was terminated by filtration onto GF/C filters presoaked with 0.05% polyethylenimine using a Tomtech call harvester and radioactivity remaining on the filters was determined as described above. Specific uptake was defined as the difference in uptake in the presence and absence of 5 μM mazindol (hDAT and hNET) or 5 μM imipramine (hSERT).

PDE4 Assay. PDE4 enzyme assays were carried out as described previously.[25] Studies with recombinant PDE4 enzymes were carried out in the laboratory of Professor Marco Conti (Stanford University, Palo Alto, CA).

Data Analysis. GraphPad Prism (GraphPad Software, San Diego, CA) was used to determine the saturation and binding kinetic data. IC_{50} values were converted to K_i values using the Cheng−Prusoff equation.

Mouse and Rat Liver Microsome Stability Assay. Microsomal incubations (final volume 0.25 mL) with the SSRIs, dual PDE4 inhibitor/SSRIs or PDE4 inhibitor was done with an HPLC assay as described above. Diluted stocks of either mouse, human, or rat liver microsomes (0.5 mg of protein), 100 μM potassium phosphate buffer (pH 7.4), 0.5 mM $NADP^+$, 0.5 mM glucose-6-phosphate, 5 IU/mL glucose-6-phosphate dehydrogenase, 0.6 mM DETAPAC, and 3 mM $MgCl_2$ were combined and placed on ice. For a metabolic stability assay, either vehicle or 40 μM test compound was added and the incubation was initiated at 37 °C with constant shaking. After 0, 10, 25, 40, and 60 min, the incubations were stopped by the addition of 1 mL CH_2Cl_2/2-propanol (3:1, v:v). After thorough mixing, the organic layer was separated from the aqueous portion by centrifugation at 12000*g*. The organic material was evaporated with a stream of argon. The residue was dissolved in methanol (200 μL), and the sample was analyzed by HPLC.

In Vivo Evaluation of Selected Compounds. Separate nonhabituated male mice were used in the conduct of this work. Fully approved animal protocols were used and the studies were done in keeping with the NIH standards for use of experimental animals.

Experiment 1. Forty male ICR mice, weighing 26.2 ± 0.3 g, were housed in a temperature-controlled room (22−23 °C) and maintained on a 12 h on/12 h off light cycle (lights on at 6:00 a.m.). Water and food were freely available in the home cages. The mice were randomly divided into four groups: (A) vehicle, (B) compound **14**, (C) compound **19**, and (D) compound **21** and administered compounds were dissolved in 0.9% saline containing 5% dimethyl sulfoxide (DMSO). Saline containing the same

percentage of DMSO was used as vehicle. The lower doses (i.e., 0.3 mg/kg for **19** and **21** or 10 mg/kg for **14**) were tested followed by the larger doses (3 mg/kg for **19** and **21** or 30 mg/kg for **14)** 1 week later, when the mice were again randomly divided into four groups as described above (Figure 1). The forced-swim test (FST) was carried out as described previously.[23] Mice were given a swimming pretest session once a day for two successive days. Then 24 hours after the last session, mice were injected with vehicle or the lower dose of each test compound by the ip route 30 min prior to the FST. One week later, administration of the larger dose of each of the test compounds was repeated. During the pretest and test sessions, each mouse was placed for 6 min in a plastic cylinder (45 cm high × 20 cm diameter), which was filled to a depth of 28 cm with water (23 ± 1 °C). The duration of immobility, which was defined as floating in an upright position without additional activity other than that necessary for the animal to keep its head above water, was recorded.[22,23] In a control experiment, acute administration of fluoxetine (40 mg/kg) or repeated treatment with rolipram (0.5 mg/kg, ip, once a day for 8 days, was required to produce similar antidepressant-like effects in the FST (data not shown).

Experiment 2. Forty Balb/c male mice weighing 22.4 ± 0.1 g were used in this experiment, which was carried out in two sessions. In each session, the mice were divided into four groups: (A) vehicle (saline containing 5% DMSO), (B) fluoxetine, 20 mg/kg, (C) compound **21**, 10 mg/kg, and (D) compound **14**, 10 mg/kg for the first session and (A) vehicle, (B) Fluoxetine, 10 mg/kg, (C) compound **14**, 10 mg/kg, and (D) compound **21**, 10 mg/kg for the second session, which was carried out 4 weeks later. Mice in the first session (Figure 2) were treated with vehicle or the test agents acutely, whereas mice in the second sessions (Figure 3) were treated subchronically, i.e., vehicle or drugs were given ip 23, 5, and 1 h before the FST. The experiment was done similar to Experiment 1 except for the pretest training, which was not carried out in the second session.

In Vivo Statistics. All in vivo data were analyzed by one-way analyses of variance (ANOVA) followed by Dunnett's tests for post hoc comparisons of individual means.

Synthesis. Ethyl-3-(2′- Methoxy-5′-fluorophenyl)-2-propenoate (2). To a solution of 2-methoxy-5-fluoro benzaldehyde (4.3 g, 28.0 mmol, 1 equiv) in dry CH_2Cl_2 (30 mL) at 0 °C was added carbethoxymethylenetriphenylphosphorane (10.7 g, 30.1 mmol, 1.1 equiv) portionwise and the reaction was warmed to room temperature and stirred for 12 h. The solvent was then removed in vacuo, and the residue was purified by flash column chromatography (EtOAc/hexane, 10:90, v:v, $R_f = 0.2$) to yield the product (6.0 g, 95%) as a 4:1 mixture of *trans*/*cis* diastereomers. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.93 (d, $J = 16.2$ Hz, 0.8 H), 7.33 (dd, $J = 3.1$, 9.2 Hz, 0.2 H), 7.21 (dd, $J = 3.1$, 9.2, Hz, 0.8 H), 7.08 (d, $J = 12.5$ Hz, 0.2 H), 7.03 (dt, $J = 3.0$, 8.8 Hz, 0.8 H), 6.99 (dt, $J = 3.0$, 8.8 Hz, 0.2 H), 6.84 (dd, $J = 3.1$, 8.6 Hz, 0.8 H), 6.79 (dd, $J = 3.3$, 9.1 Hz, 0.2 H), 6.47 (d, $J = 16.2$ Hz, 0.8 H), 5.99 (d, $J = 12.5$ hz, 0.2 H), 4.25 (q, $J = 7.1$ Hz, 1.6 H), 4.15 (q, $J = 7.0$ Hz, 0.4 H), 3.86 (s, 2.4 H), 3.81 (s, 0.6 H), 1.34 (t, $J = 7.1$ Hz, 2.4 H), 1.22 (t, $J = 7.0$ Hz, 0.6 H).

Ethyl-4-(5′-fluoro-2′-methoxyphenyl)propanoate (3). To a solution of **2** (6.0 g, 27.0 mmol, 1 equiv) in ethanol (200 proof, 40 mL) under Ar was added Pd/C (10%, 250 mg, 0.1 equiv), and the flask containing the mixture was evacuated and purged with H_2 three times. H_2 was attached to the flask and the reaction was allowed to stir for 15 h at room temperature. The reaction mixture was filtered through a pad of silica and eluted with EtOAc/hexane, 50:50, v:v. The crude product obtained by removal of the solvent in vacuo was purified by a flash column chromatography (EtOAc/hexane, 10:90, v:v, $R_f = 0.27$) to give the product (5.8 g, 95%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.89−6.84 (m, 2 H), 6.74 (dd, $J = 4.7$, 9.8 Hz, 1 H), 4.12 (q, $J = 7.1$ Hz, 2 H), 3.79 (s, 3 H), 2.91 (t, $J = 7.7$ Hz, 2 H), 2.59 (t, $J = 7.7$ Hz, 2 H), 1.24 (t, $J = 7.2$ Hz, 3 H).

3-(5′-Fluoro-2′-methoxyphenyl)propanal (4). To a solution of **3** (5.8 g, 25.7 mmol, 1 equiv) in dry toluene (40 mL) under an atmosphere of Ar was added a DIBAL solution (1 M in toluene, 30 mL, 30 mmol, 1.2 equiv) at −78 °C. The reaction was stirred at −78 °C for 2 h. Methanol (2 mL) was then added to the mixture, and the reaction was allowed to warm to 0 °C. The reaction mixture was then poured into a separatory funnel containing HCl solution (1 N, 100 mL). The organic fraction was extracted with EtOAc (3 × 60 mL), and the combined organic layers were washed with brine (80 mL) and dried over sodium sulfate. The crude product obtained by removal of the solvent in vacuo was purified by flash column chromatography (EtOAc/hexane, 10:90, v:v, $R_f = 0.1$) to give the product (3.1 g, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.80 (s, 1 H), 6.88−6.86 (m, 2 H), 6.75 (dd, $J = 4.6$, 8.6 Hz, 1 H), 3.79 (s, 3 H), 2.91 (t, $J = 7.4$ Hz, 2 H), 2.72 (t, $J = 7.4$ Hz, 2 H).

1-(5′-Fluoro-2′-methoxyphenyl)-4-ethylenedioxy-1-butene (5). To a suspension of 2-(1,3-dioxolan-2-yl)ethyltriphenylphosphonium bromide (5.0 g, 11.3 mmol, 1 equiv) in THF (30 mL) under an atmosphere of Ar was added NaH (60% in mineral oil, 0.48 g, 11.3 mmol, 1 equiv), and the mixture obtained was heated to reflux for 1 h. The orange suspension obtained was cooled to 0 °C and 5-fluoro-2-methoxybenzaldehyde (1.54 g, 10 mmol, 0.9 equiv) was added and the reaction was warmed to room temperature and continued with stirring for 12 h. The reaction mixture was poured into a separatory funnel containing ammonium chloride aqueous solution (sat. NH_4Cl/H_2O 50:50, v:v, 50 mL). The organic material was extracted with EtOAc (3 × 80 mL), and the combined organic layers were washed with brine (60 mL) and dried over sodium sulfate. The solvent was then removed in vacuo and the crude product obtained was purified by flash column chromatography (EtOAc/hexane, 7:93, v:v, $R_f = 0.15$) to give the product (2.63 g, 66%) as a 4:1 mixture of *cis* and *trans* diastereomers. ^{1}H NMR (major isomer) ($CDCl_3$, 500 MHz): δ 7.08 (dd, $J = 3.1$, 8.7 Hz, 1 H), 6.93 (dt, $J = 3.1$, 8.7 Hz, 1 H), 6.78 (dd, $J = 4.3$, 8.7 Hz, 1 H), 6.63 (d, $J = 11.9$ Hz, 1 H), 5.83 (td, $J = 7.3$ Hz, 11.9 Hz, 1 H), 4.99 (t, $J = 4.6$ Hz, 1 H), 4.02−3.99 (m, 2 H), 3.9−3.87 (m, 2 H), 3.8 (s, 3 H), 2.64−2.62 (m, 2 H).

4-(5′-Fluoro-2′-methoxyphenyl)-1-ethylenedioxybutane (6). To a solution of **5** (1.75 g, 7.3 mmol, 1 equiv) in ethanol (200 proof, 80 mL) under an atmosphere of Ar was added Pd/C (10%, 300 mg, 0.1 equiv), and the flask containing the mixture was evacuated and purged with H_2 three times. H_2 gas was attached to the flask and the reaction was allowed to proceed for 72 h at room temperature. The reaction mixture was then filtered through a pad of celite and eluted with EtOH. The crude product was obtained by removal of the solvent in vacuo. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.87−6.72 (m, 3 H), 4.87 (t, $J = 3.8$ Hz, 1 H), 3.97−3.95 (m, 2 H), 3.86−3.83 (m, 2 H), 3.79 (s, 3 H), 2.64−2.62 (m, 2 H), 2.44 (td, $J = 1.7$, 7.2 Hz, 2 H), 1.92 (quintet, $J = 7.5$ Hz, 2 H).

4-(5′-Fluoro-2′-methoxyphenyl)butyraldehyde (7). To a solution of **6** (1.7 g, 7.0 mmol, 1 equiv) in THF (60 mL) was added HCl (1 N, 3 mL), and the reaction was stirred at room temperature for 48 h. The reaction mixture was then poured into a separatory funnel containing water (60 mL). The organic fraction was extracted with EtOAc (3 × 60 mL), and the combined organic layers were washed with brine (80 mL) and dried over sodium sulfate. The crude product obtained by removal of the solvent in vacuo was purified by flash column chromatography (EtOAc/hexane, 10:90, v:v, $R_f = 0.1$) to give the product (1.03 g, 53% from **3a**). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.76 (s, 1 H), 6.88−6.83 (m, 2 H), 6.75 (dd, $J = 4.6$, 8.6 Hz, 1 H), 3.79 (s, 3 H), 2.63 (t, $J = 7.4$ Hz, 2 H), 2.44 (t, $J = 7.4$ Hz, 2 H), 1.92 (quintet, $J = 7.4$ Hz, 2 H).

7-(5′-Fluoro-2′-methoxyphenyl)hept-1-en-5-ol (8). To a solution of **4** (3.0 g, 19.5 mmol, 1 equiv) in THF (20 mL) at 0 °C was added a solution 1-butenylmagnesium bromide (0.5 M in THF, 45 mL, 22.5 mmol, 1.1 equiv) dropwise for 15 min. The reaction mixture was poured into a solution of saturated ammonium chloride (80 mL) in a separatory funnel. The organic fraction was extracted with EtOAc (3 × 60 mL) and the combined organic layers were washed with brine (50 mL) and dried over sodium sulfate. The product was purified by flash column chromatography (EtOAc/hexane, 15:85, v:v, $R_f = 0.2$) to give the product as a colorless oil (3.7 g, 94%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.86−6.82 (m, 2 H),

6.76 (dd, J = 4.6, 8.6 Hz, 1 H), 5.82 (m, 1 H), 5.04 (d, J = 17.3 Hz, 1 H), 4.95 (d, J = 10.2 Hz, 1 H), 3.81 (s, 3 H), 3.55 (m, 1 H),2.75−2.69 (m, 2 H), 2.2 (m, 1 H), 2.1 (m, 1 H), 1.96 (bs, 1 H), 1.73−1.68 (m, 2 H), 1.6−1.53 (m, 3 H).

The following compound was prepared in a similar manner.

8-(5′-Fluoro-2′-methoxyphenyl)oct-1-en-5-ol (9). The product was purified by flash column chromatography (EtOAc/hexane, 12.5:87.5, v:v, R_f = 0.15) as a colorless oil (1.06 g, 61%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.86−6.82 (m, 2 H), 6.74 (dd, J = 4.6, 8.6 Hz, 1 H), 5.85 (m, 1 H), 5.04 (d, J = 17.3 Hz, 1 H), 4.97 (d, J = 10.2 Hz, 1 H), 3.79 (s, 3 H), 3.66 (m, 1 H), 2.6 (m, 2 H), 2.22−2.1 (m, 2 H), 1.74−1.47 (m, 7 H).

2-(Bromomethyl)-5-(2′-methoxy-5′-fluorophenethyl)tetrahydrafuran (10). To a solution of **8** (3.24 g, 18.0 mmol, 1 equiv) in dry CH_2Cl_2 (50 mL) at 0 °C was added *N*-bromosuccinimide (3.56 g, 20 mmol, 1.1 equiv) portionwise, and the reaction was warmed to room temperature and stirred for 12 h. Solvent was then removed in vacuo and the product was purified by flash column chromatography (EtOAc/hexane, 5:95, v:v, R_f = 0.1) and resulted in a colorless oil as a 2:1 mixture of *trans*/*cis* diastereomers. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.86−6.81 (m, 2 H), 6.72 (dd, J = 4.6, 8.6 Hz, 1 H), 4.22 (m, 1 H), 4.03 (m, 1 H), 3.78 (s, 3 H), 3.44 (dd, J = 4.3, 9.7 Hz, 1 H), 3.34 (dd, J = 6.4, 9.7 Hz, 1 H), 2.6 (t, J = 7.3 Hz, 2 H), 2.13 (m, 1 H), 2.05 (m, 1 H), 1.75 (m, 1 H), 1.67−1.46 (m, 5 H).

The following compound was prepared in a similar manner.

2-(Bromomethyl)-5-(3′-(2″-methoxy-5″-fluorophenyl)-1′-propyl)tetrahydrofuran (11). The product was purified by flash column chromatography (EtOAc/hexane, 5:95, v:v, R_f = 0.1) to provide a product (530 mg, 40%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.86−6.81 (m, 2 H), 6.72 (dd, J = 4.6, 8.6 Hz, 1 H), 4.22 (m, 1 H), 4.03 (m, 1 H), 3.78 (s, 3 H), 3.44 (dd, J = 4.3, 9.7 Hz, 1 H), 3.34 (dd, J = 6.4, 9.7 Hz, 1 H), 2.6 (t, J = 7.3 Hz, 2 H), 2.13 (m, 1 H), 2.05 (m, 1 H), 1.75 (m, 1 H), 1.67−1.46 (m, 5 H).

2-Cyanomethyl-5-(5′-fluoro-2-methoxyphenethyl)tetrahydrofuran (12). To a vial under an atmosphere of $Ar_{(g)}$ was added **10** (1.23 g, 4.3 mmol, 1 equiv), NaI (100 mg, 1.3 mmol, 0.3 equiv), potassium cyanide (0.7 g, 10.6 mmol, 2.4 equiv), and dry DMSO (15 mL). The mixture obtained was heated to 70 °C under an atmosphere of Ar for 12 h. After cooling to room temperature, the reaction mixture was poured into a separatory funnel containing sodium bicarcarbonate aqueous solution (sat. $NaHCO_3/H_2O$, 50:50, v:v, 80 mL). The organic fraction was extracted with EtOAc (3 × 60 mL), and the combined organic layers were washed with brine (50 mL) and dried over sodium sulfate. The solvent was then removed in vacuo and the crude product obtained (0.45 g, 86%) was purified by flash column chromatography (EtOAc/hexane, 10:90, v:v, R_f = 0.11) as a 2:1 mixture of *trans*/*cis* diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88−6.82 (m, 2 H), 6.74 (dd, J = 4.3, 8.5 Hz, 1 H), 4.26 (m, 1 H), 4.09 (m, 0.65 H), 3.90 (m, 0.35 H)3.79 (s, 3 H), 2.72−2.53 (m, 4 H), 2.22−2.04 (m, 2 H), 1.84−1.6 (m, 4 H).

The following compound was prepared in a similar manner.

2-Cyanomethyl-5-(3′-(2″-methoxy-5″-fluorophenyl)-1′-propyl)-tetrahydrafuran (13). The product (0.2 g, 80%) was purified by chromatography (EtOAc/hexane, 10:90, v:v, R_f = 0.11) to give a 2:1 mixture of *trans*/*cis* diastereomers. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.86−6.81 (m, 2 H), 6.73 (dd, J = 4.2, 8.5 Hz, 1 H), 4.22 (p, J = 6.8 Hz, 0.65 H), 4.11 (m, 0.35 H) 4.09 (m, 0.65 H), 3.9 (m, 0.35 H), 3.79 (s, 3 H), 2.62−2.52 (m, 4 H), 2.18 (m, 1 H), 2.12 (m, 1 H), 1.78 (m, 1 H), 1.69−1.46 (m, 3 H).

2-{5-[3-(5-Fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine (14). To a round-bottom flask under an atmosphere of Ar was added Raney Ni (5 mg, 0.03 mmol, 0.1 equiv) that was washed with methanol. Compound **12** (80 mg, 0.3 mmol, 1 equiv), dissolved in 2 M NH_3 in methanol, was added to the flask. The flask was evacuated and purged with H_2 once. H_2 gas was then attached to the flask, and the reaction was allowed to stirr for 4 h at room temperature. The reaction mixture was filtered through a plug of celite and the crude product obtained after the removal of the solvent was purified by preparative TLC and eluted with ($MeOH/CH_2Cl_2$, 10:90, v:v, R_f = 0.1) to give the product (55 mg, 70%) as a 2:1 mixture of *trans*/*cis* diastereomers. ^{1}H NMR: ($CDCl_3$, 500 MHz): δ 6.87 (m, 1 H), 6.82 (dd, J = 3.0, 8.5 Hz, 1 H), 6.72 (m, 1 H), 4.03 (m, 0.65 H), 3.96 (m, 0.65 H), 3.89 (m, 0.35 H), 3.82 (m, 0.35 H), 3.78 (s, 3 H), 2.68 (m, 1 H), 2.58 (m, 1 H), 2.03 (m, 2 H), 1.83 (bs, 2 H), 1.86−1.6 (m, 4 H), 1.54 (m, 2 H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{23}FNO_2$ 268.1713, found 268.1701.

The following compound was prepared in a similar manner.

2-{5-[3-(5-Fluoro-2-methoxy-phenyl)-propyl]-tetrahydro-furan-2-yl}-ethylamine (15). The product (24 mg, 47%), was isolated as 3:1 mixture of *trans*/*cis* diastereomers ($MeOH/CH_2Cl_2$, 10:90, v:v, R_f = 0.1). ^{1}H NMR: ($CDCl_3$, 500 MHz): δ 6.86−6.8 (m, 2 H), 6.73 (m, 1 H), 4.02 (m, 0.65 H), 3.96 (m, 0.65 H), 3.92 (m, 0.35 H), 3.82 (m, 0.35 H), 3.78 (s, 3 H), 3.09 (m, 1 H), 2.99 (m, 1 H), 2.6 (bs, 2 H), 1.96 (m, 2 H), 1.83−1.77 (m, 2 H), 1.65−1.47 (m, 8 H). HRMS (ESI) $[M + H]^+$ calcd for $C_{16}H_{25}FNO_2$ 282.1875, found 282.1869.

3,4-Dimethoxyphenylmagnesium bromide (17). Into a flame-dried round-bottom flask with a magnetic stir bar was placed anhydrous THF (150 mL) and $Mg_{(s)}$ turnings (1.43 g, 58.8 mmol, 1.0 equiv). The flask was then fitted with a pressure equalizing addition funnel containing 4-bromo-1,2-dimethoxybenzene (8.2 g, 58.8 mmol, 1 equiv) (**16**) solution that was added slowly over a period of 45 min while stirring at rt. Once the addition was complete, $I_{2(s)}$ (500 mg) was added and the reaction was brought to reflux and stirred for 12 h. The Grignard reagent was cooled and used immediately in the following reaction.

6-(3,4-Dimethoxy-benzoyl)-cyclohex-3-enecarboxylic acid (18). A solution of **17** (9.6 g, 59 mmol, 0.29 M, 1 equiv) in THF was added dropwise to an ice-cooled solution of *cis*-1,2,3,6-tetrahydrophthalic anhydride (8.9 g, 59 mmol, 1 equiv) in THF (120 mL) over a 1 h period. After the addition was complete, the resulting mixture was stirred for another 30 min at 0 °C. The reaction mixture was allowed to warm to rt and stirred overnight. The reaction was then stopped with sat. NH_4Cl and the pH adjusted to 2 with concentrated $HCl_{(aq)}$ and extracted with diethyl ether. The organic layer was washed with water and subsequently extracted with 1 M NaOH. The combined aqueous extract was acidified with concentrated HCl and extracted with EtOAc (3 × 100 mL). The organic layers were combined and dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford oil. The oil was dissolved in CH_2Cl_2 and filtered through silica gel to remove the dicarboxylic acid formed during workup. The product was recrystallized from diethyl ether to afford pure product (1.62 g, 10%) as a white solid; mp = 109 °C. LRMS ESI $[M - H]^-$ calcd for $C_{16}H_{18}O_5$ 289, found m/z 289. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.60−7.53 (m, 2 H), 6.92 (d, J = 8.4 Hz, 1 H), 5.84−5.82 (m, 1 H), 5.71−5.68 (m, 1 H), 4.03−4.00 (m, 1 H), 3.98 (s, 3 H), 3.95 (s, 1 H), 3.10−3.07 (m, 1 H), 2.90−2.84 (m, 1 H), 2.54−2.44 (m, 3 H).

4-(3,4-Dimethoxy-phenyl)-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (19). A mixture of **18** (610 mg, 2.1 mmol, 1.0 equiv) and hydrazine hydrate (168 mg, 5.25 mmol, 2.5 equiv) in EtOH (10 mL) was refluxed for 4 h. The reaction was then cooled to rt and concentrated under reduced pressure to afford a white precipitate. The precipitate was dissolved in EtOAc and washed with $Na_2SO_{4(aq)}$, 1 N $HCl_{(aq)}$, and water. The organic layer was then dried over $MgSO_4$, filtered, and concentrated to give a white precipitate. The precipitate was recrystalized in EtOH to afford the product as white crystals (376 mg, 63%). HRMS ESI $[M + H]^+$ calcd for $C_{16}H_{18}N_2O_3$ 287.3385, found m/z 287.3379. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.53 (bs, 1 H), 7.46 (d, J = 2.0 Hz, 1 H), 7.23 (dd, J = 2.0, 8.4 Hz, 1 H), 6.87 (d, J = 8.4 Hz, 1 H), 5.8−5.77 (m, 1 H), 5.72−5.7 (m, 1 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.4 (dt, J = 5.5, 8.7 Hz, 1 H), 3.01−2.97 (m, 1 H), 2.85 (t, J = 6.0 Hz, 1 H), 2.26−2.19 (m, 3 H). HRMS (ESI) $[M + H]^+$ calcd for $C_{16}H_{19}N_2O_3$ 287.3385, found 287.3376. HPLC > 98% pure (t_R = 4.93, 70(A):30(E); t_R = 5.33, 50(A):50(E)).

2-(5-Bromo-pentyl)-4-(3,4-dimethoxy-phenyl-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (20). Sodium hydride (60% dispersion in oil, 44 mg, 1.1 mmol, 1.1 equiv) was added to a solution **19** (287 mg, 1.0 mmol, 1.0 equiv). The mixture was stirred at rt for 30 min, whereupon it took on a slight yellow color. 1,5-Dibromopentane (600 mg, 2.6 mmol, 2.6 eq) was added via syringe, and the reaction was stirred for 30 min at rt while the yellow color dissipated to an almost clear solution. The reaction was stopped with the addition of water and then transferred to a separatory funnel and extracted with Et_2O (3 × 30 mL). The organic fractions were combined and dried over $MgSO_4$, filtered, and concentrated to afford an oil. The product was purified on silica and eluted with CH_2Cl_2 to afford a clear oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.47 (d, J = 2.0 Hz, 1 H), 7.25 (dd, J = 2.0, 8.5 Hz, 1 H), 6.87 (d, J = 8.5 Hz, 1 H), 5.8−5.77 (m, 1 H), 5.7−5.67 (m, 1 H), 4.01−3.97 (m, 1 H), 3.95 (s, 3 H), 3.92 (s, 3 H), 3.79−3.74 (m, 1 H), 2.22−2.18 (m, 2 H), 2.1−2.04 (m, 1 H), 1.94−1.86 (m, 4 H), 1.74−1.69 (m, 4 H), 1.63−1.56 (m, 1 H), 1.52−1.46 (m, 2 H). LRMS ESI $[M + H]^+$ calcd for $C_{21}H_{28}BrN_2O_3$ 436, found *m*/*z* 435 (Br^{79}), 437 (Br^{81}). HRMS (ESI) $[M + H]^+$ calcd for $C_{21}H_{28}BrN_2O_3$ 436.8007, found 436.8025.

2-{5-[3-(5-Fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine)-pentyl]-4,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (21). Into a flame-dried round-bottom flask under an atmosphere of $Ar_{(g)}$, cesium hydroxide monohydrate (23 mg, 0.14 mmol, 1.0 equiv) and DMF (0.5 mL) was stirred for 30 min at rt. Compound **14** (37 mg, 0.14 mmol, 1.0 equiv) in DMF (0.3 mL) was then added via syringe and the resulting mixture was stirred at rt. After stirring an additional 30 min, compound **20** (72 mg, 0.16 mmol, 1.2 eq) dissolved in DMF (0.3 mL) was added and stirred for an additional 18 h at rt. The mixture was washed with EtOAc (30 mL) and then filtered, and the filtrate was washed with water in a separatory funnel. The organic layer was dried with Na_2SO_4, filtered, and concentrated to oil. The product was purified by preparative TLC (developed with MeOH/CH_2Cl_2, 5:95, v:v, R_f = 0.2) to afford an oil (27 mg, 0.04 mmol, 31%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.47 (d, J = 2.0 Hz, 1 H), 7.25 (dd, J = 2.0, 8.5 Hz, 1 H), 6.87 (d, J = 8.5 Hz, 1 H), 6.86−6.81 (m, 2 H), 6.76−6.72 (m, 1 H), 5.81−5.79 (m, 1 H), 5.71 (m, 1 H), 4.01−3.97 (m, 1 H), 3.94 (s, 3 H), 3.91 (s, 3 H), 3.8 (s, 3 H), 3.79−3.74 (m, 1 H), 3.36−3.31 (m, 1 H), 3.03−3.0 (m, 1 H), 2.81−2.78 (m, 2 H), 2.73−2.37 (m, 5 H), 2.30−2.19 (m, 3 H), 2.09−1.97 (m, 3 H), 1.87−1.26 (m, 14 H). HRMS (ESI) $[M + H]^+$ calcd for $C_{36}H_{49}FN_3O_5$ 622.8007, found 622.8025.

The following compound was prepared in a similar manner.

2-{5-[3-(5-Fluoro-2-methoxy-phenyl)-propyl]-tetrahydro-furan-2-yl}-ethylamine)-pentyl-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-one (22). The product was isolated by preparative TLC and (developed with MeOH/CH_2Cl_2, 5:95, v:v, R_f = 0.2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.47 (d, J = 2.0 Hz, 1 H), 7.25 (dd, J = 2.0, 8.5 Hz, 1 H), 6.87 (d, J = 8.5 Hz, 1 H), 6.86−6.81 (m, 2 H), 6.76−6.72 (m, 1 H), 5.8−5.77 (m, 1 H), 5.69−5.66 (m, 1 H), 4.01−3.97 (m, 1 H), 3.94 (s, 3 H), 3.91 (s, 3 H), 3.77 (s, 3 H), 3.79−3.74 (m, 1 H), 3.33−3.3 (m, 1 H), 3.0−2.96 (m, 1 H), 2.81−2.57 (m, 7 H), 2.22−2.17 (m, 3 H), 2.04−1.34 (m, 19 H). HRMS (ESI) $[M + H]^+$ calcd for $C_{37}H_{51}FN_3O_5$ 636.8275, found 636.826.

2-{5-[3-(5-Fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine)-carbamic Ethyl Ester (23). Into a flame-dried 20 mL vial was placed K_2CO_3 (239 mg, 1.73 mmol, 6.0 equiv) and anhydrous THF (5.0 mL). The vial was purged with $Ar_{(g)}$ and then cooled in an ice bath. Ethyl chloroformate (156 mg, 1.44 mmol, 5.0 equiv) was then added via syringe followed by slow addition of **14** (77 mg, 0.29 mmol, 1.0 equiv) dissolved in THF (1.5 mL). The reaction was stirred at 0 °C for 0.5 h and then warmed to rt and allowed to stir an additional 3 h. The reaction was then stopped with sat. $NaHCO_{3(aq)}$ and extracted with EtOAc (3 × 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield crude product that was used in the next step of the synthesis. TLC: MeOH/CH_2Cl_2 (2:98, v:v), R_f 0.4. ^{1}H NMR ($CDCl_3$, 500 MHz) 6.87−6.82 (m, 2 H), 6.74−6.71 (m, 1 H), 4.12 (q, J = 7.1 Hz, 2 H), 4.05−3.77 (m, 2 H), 3.78 (s, 3 H), 2.71−2.56 (m, 2 H), 2.08−1.51 (m, 10 H) 1.3 (t, J = 7.2 Hz, 3 H). LRMS (ESI) $[M + H]^+$ calcd for $C_{18}H_{27}FNO_4$ 340, found 340.

2-{5-[3-(5-Fluoro-2-methoxy-phenyl)-ethyl]-tetrahydro-furan-2-yl}-ethylamine)-methyl-amine (24). Into a flame-dried round-bottom flask, purged with $Ar_{(g)}$, was placed **23** (112 mg, 0.33 mmol, 1.0 equiv) and anhydrous THF (1.6 mL). The flask was cooled with an ice bath and stirred for 15 min. LAH (1.0 M, 1.32 mL, 1.32 mmol, 4.0 equiv) was then slowly added via a syringe over a period of 5 min. The reaction was allowed to warm to rt and stirred an additional 4 h. The reaction was then stopped with ice-cold MeOH and then stirred for an additional 15 min at rt. The resulting solution was acidified with 1 N $HCl_{(aq)}$ and then transferred to a beaker and then made basic with 10 M $NaOH_{(aq)}$. The basic solution was extracted with diethyl ether (3 × 20 mL), and then the organic layer was washed with brine. The diethyl ether extract was dried over Mg_2SO_4 for 15 min and then filtered and concentrated to oil under reduced pressure to afford the crude product. The oil was purified with preparative TLC (developed with MeOH/CH_2Cl_2, 5:95, v:v, R_f 0.05). ^{1}H NMR ($CDCl_3$, 500 MHz) 6.87−6.82 (m, 2 H), 6.74−6.71 (m, 1 H), 4.05−3.77 (m, 2 H), 3.78 (s, 3 H), 2.71−2.56 (m, 5 H), 2.08−1.51 (m, 10 H). HRMS (ESI) $[M + H]^+$ calcd for $C_{16}H_{25}FNO_2$ 282.3784, found 282.3762.

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Supporting Information Available: Analytical data of the target compounds is provided in the Supporting Information. This information is available free of charge via the Internet at http://pubs.acs.org.

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