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Bicyclic Diazepinones as Dual Ligands of the α2δ-1 Subunit of Voltage-Gated Calcium Channels and the Norepinephrine Transporter

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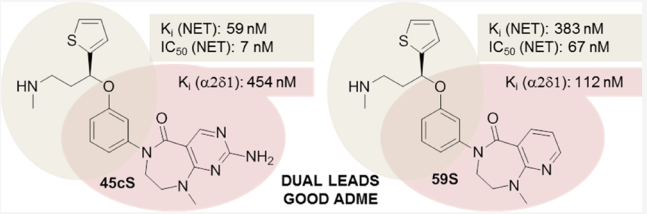
ABSTRACT: The synthesis and pharmacological activity of a new series of bicyclic diazepinones with dual activity toward the α2δ-1 subunit of voltage-gated calcium channels ($Ca_v\alpha2\delta$-1) and the norepinephrine transporter (NET) are reported. Exploration of the positions amenable for substitution on a nonaminoacidic $Ca_v\alpha2\delta$-1 scaffold allowed the identification of favorable positions for the attachment of NET pharmacophores. Among the patterns explored, attachment of the 2-ethylamino-9-methyl-6-phenyl-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*e*][1,4]diazepin-5-one framework to the meta-position of the phenyl ring of the 3-methylamino-1-phenylpropoxy and 3-methylamino-1-thiophenylpropoxy moieties provided dual compounds with excellent NET functionality. Alternative bicyclic frameworks were also explored, and some lead molecules were identified, which showed a balanced dual profile and exhibited good ADMET properties.



INTRODUCTION

The treatment of pain constitutes an important challenge since current therapies are suboptimal and hampered by side effects, which contribute to noncompliance or limited efficacy.[1,2] Considering that most pain states involve multiple mediators and signaling pathways, both central and peripheral, multimodal analgesic approaches are often used in the clinical practice[3] and recommended in management guidelines. Additive or synergistic effects between interventions can enhance analgesia, and efficacy may be achieved at lower doses, potentially reducing the risk of adverse effects.[4] Combinations of available drugs are often used in the clinic,[5] but developing single compounds with multimodal activity may offer some advantages, such as better efficacy, superior treatment compliance (one instead of various pills at potentially different dosage intervals/frequencies), lowering the risk of drug−drug interactions, simpler pharmacokinetics, and less variability among patients, both in drug exposure and response to treatment.[6] With this view in mind, we decided to explore the preparation of dual ligands acting at the α2δ-1 subunit of voltage-gated calcium channels ($Ca_v\alpha2\delta$-1) and at the norepinephrine (NE) transporter (NET, SLC6A2), two mechanisms with clear rationale in the treatment of pain.

Voltage-gated calcium channels (VGCC)[7] are required for many key functions in the body and are assembled through interactions of different subunits, namely, α_1 ($Ca_v\alpha_1$), β ($Ca_v\beta$), $\alpha_2\delta$ ($Ca_v\alpha_2\delta$), and γ ($Ca_v\gamma$). The α_1 subunits are the key porous forming units of the channel complex, regulating Ca^{2+} conduction and influx. The $\alpha_2\delta$, β, and γ subunits are auxiliary but key for increasing the expression of the α_1 subunits in the plasma membrane and modulating their function. VGCC are subdivided into five different subtypes: low voltage-activated T-type ($Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$) and high voltage-activated L-type ($Ca_v1.1$ through $Ca_v1.4$), N-type ($Ca_v2.2$), P/Q-type ($Ca_v2.1$), and R-type ($Ca_v2.3$). Both the Ca_v1 and Ca_v2 subfamilies contain an auxiliary α2δ subunit,[8] which is also subdivided in four different subtypes: α2δ-1, initially cloned from skeletal muscle and showing a fairly ubiquitous distribution; α2δ-2 and α2δ-3 subunits, cloned from brain; and α2δ-4, recently cloned and largely non-neuronal.

The $Ca_v\alpha2\delta$-1 subunit (and the $Ca_v\alpha_2\delta$-2 but not $Ca_v\alpha_2\delta$-3 and $Ca_v\alpha_2\delta$-4 subunits) is the therapeutic target[9] of pregabalin (**1**) and gabapentin (**2**), two widely used drugs (Figure 1) for treating neuropathic pain[10] and fibromyalgia[11] as well as other

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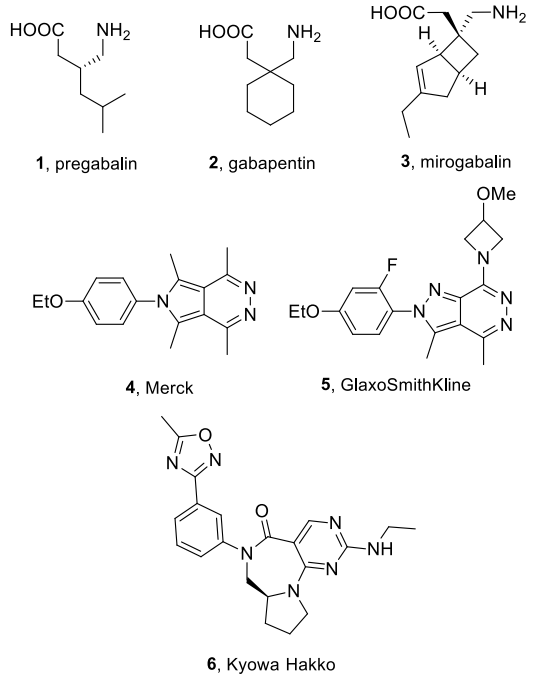

Figure 1. Structure of described blockers of the $Ca_v\alpha2\delta$-1.

nonpain indications, such as epilepsy[12] or restless leg syndrome.[13] The binding of these drugs to the $Ca_v\alpha2\delta$-1 subunit results in a reduction in the calcium-dependent release of multiple neurotransmitters, and its upregulation in the spinal dorsal horn and dorsal root ganglia after nerve injury correlates with neuropathic pain development.

An important effort has been devoted along the years to finding new $Ca_v\alpha2\delta$-1 ligands for the treatment of pain.[14] Most of the described compounds have aminoacidic structures closely related to **1** and **2**, and mirogabalin (**3**)[15,16] has been the only newly approved compound in more than 15 years. A few nonaminoacidic structures have also been reported, but none of them have progressed into clinical development. Among others,[17,18] Merck reported several $Ca_v\alpha2\delta$-1 ligands represented by compound **4**,[19−21] a structure that was later modified by GlaxoSmithKline[22] to afford compounds such as **5**, which showed good analgesic activity in the complete Freund's adjuvant pharmacodynamic model of pain. Finally, Kyowa Hakko Kirin Co., filed two patent families,[23,24] describing pyrimidodiazepinone tricyclic derivatives represented by compound **6**.

On the other hand, NET is a monoamine transporter mostly expressed in the peripheral and central nervous systems, which recycles primarily NE (but also serotonin and dopamine) from synaptic spaces into presynaptic neurons. It is closely linked to the serotonin transporter (SERT), which exerts similar functions with serotonin. NET inhibition provides an increased availability of NE for binding to postsynaptic receptors that regulate adrenergic neurotransmission. Numerous studies have demonstrated that activation of spinal $\alpha2$-adrenergic receptors exerts a strong antinociceptive effect[25] and the tricyclic antidepressants (TCAs), represented by amitriptyline, have been used in the treatment of chronic pain for decades.[26] The TCAs are nonselective for NET and are the basis for the development of more specific inhibitors that modulate both the noradrenergic and the serotonergic systems with different degrees of selectivity, giving rise to selective serotonin reuptake inhibitors (SSRIs, represented by fluoxetine, **7**), selective NE reuptake inhibitors (NRIs, represented by reboxetine, **8**, and atomoxetine, **9**), and the dual inhibitors of SERT and NET (SNRIs, represented by duloxetine, **10**). This compound class is primarily used for the treatment of depression and other psychiatric disorders but has also relevant effects in the treatment of pain, mainly attributed to the NET.[27] In fact, the NRI reboxetine (**8**) has been shown to reverse allodynia in animal models[28] and the SNRI duloxetine (**10**) produces a decrease in pain sensitivity, whereas the SSRI, fluoxetine (**7**), is ineffective. Duloxetine has been approved for the treatment of diabetic neuropathy, fibromyalgia, and chronic musculoskeletal pain by the Food and Drug Administration (FDA), reinforcing the involvement of monoamine transporters in pain etiology.[29]

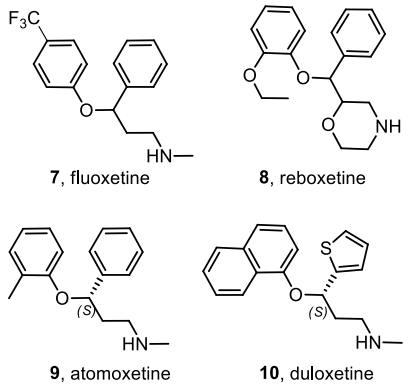

Figure 2. Structures of representative SSRI (fluoxetine), NRI (reboxetine and atomoxetine), and SNRI (duloxetine).

In the search for dual ligands acting at the $Ca_v\alpha2\delta$-1 and NET, we decided to focus on the nonaminoacidic $Ca_v\alpha2\delta$-1 ligands since the SAR data published on the aminoacidic structures indicated that achieving relevant activity was restricted to a narrow structural framework. The pyrimidodiazepinone nucleus of compound **6** was selected for fusing or merging with the more homogeneous NET pharmacophore, common to compounds **7**−**10**. Although several approaches have been used for the design of dual compounds,[6,30] such as mutual prodrugs[31] or bivalent ligands,[32] the best scenario for achieving the desired drug-like properties is provided by "adding" the pharmacophores required for the individual activities or even better "merging" them by taking advantage of commonalities in their structures. This was not an obvious task since both target pharmacophores did not seem to have much in common, but as depicted in Figure 3, we hoped that this strategy would allow the identification of dual compounds that showed advantages over the individual therapies and their mere combination. We report here the structure−activity relationship (SAR) studies that led to the identification of a new series of bicyclic diazepinones[33,34] with dual affinity toward the $Ca_v\alpha2\delta$-1 and NET.

RESULTS AND DISCUSSION

Chemistry. The synthesis of the compounds described herein (Tables 1, 2, and 3) was accomplished, as depicted in Schemes 1−5. The most simple oxadiazolyl derivatives (Table 1) were prepared from methylthiopyrimidyl starting materials, as shown in Scheme 1. Pyrimidine intermediates **13** were obtained by SNAr amination of chloropyrimidine **11** with methyl or ethyl ethylenediamine (**12**) in the presence of a

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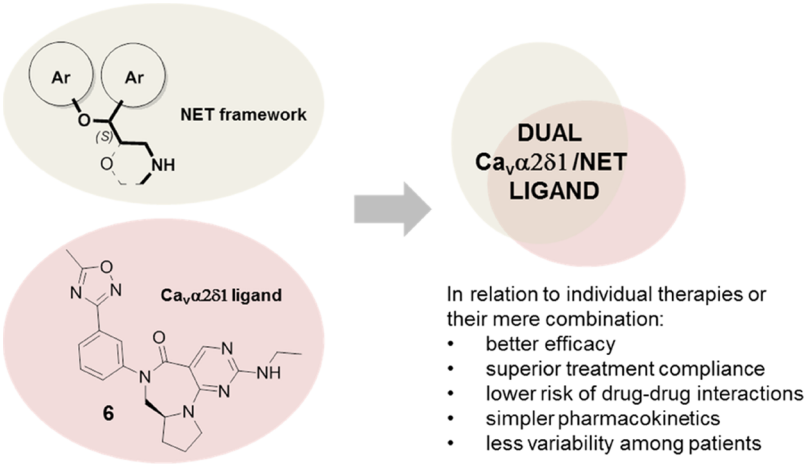
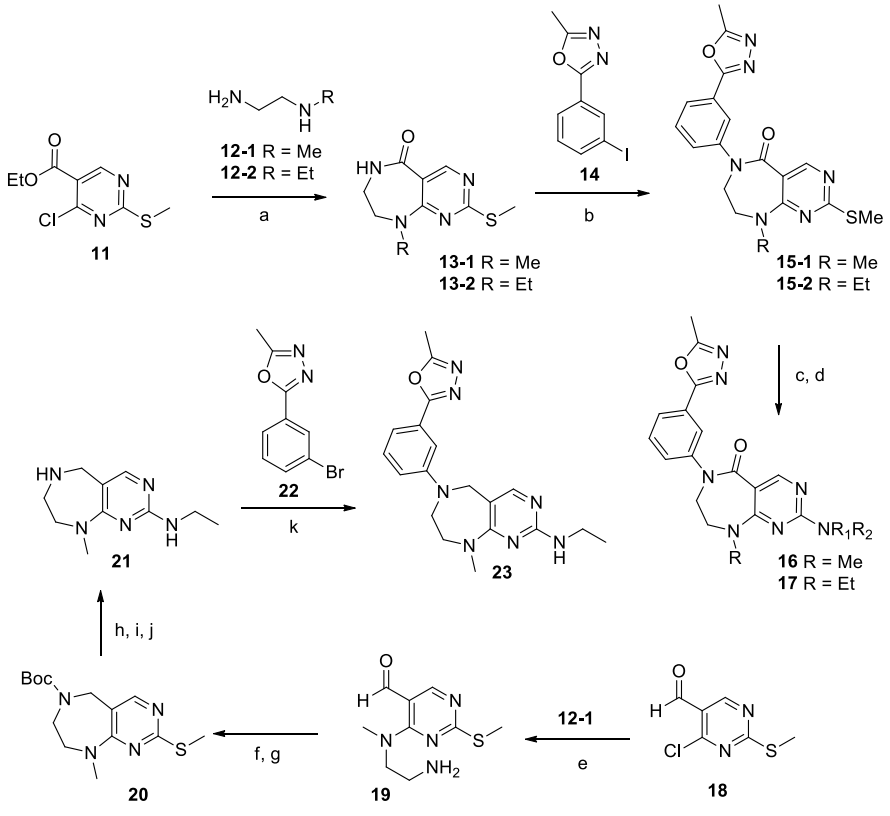


Figure 3. Strategy for developing dual Ca$_v$$\alpha$2$\delta$-1 and NET ligands.

Scheme 1. Preparation of compounds 15, 16, 17 and 23.[a]



[a]Reagents and conditions: (a) for R = Me: KO^{t}Bu, THF, rt, and 24 h; for R = Et: Et_3N, ACN, 80 °C, and 48 h; (b) CuI, K_3PO_4, N^1,N^2-dimethylethane-1,2-diamine, dioxane, 100 °C, and 16 h; (c) *m*-CPBA, DCM, rt, and 1 h; (d) NHR_1R_2, THF, rt, and 16 h; (e) Et_3N, ACN, 0 °C to rt, and 20 h; (f) $NaBH(OAc)_3$, DCE, rt, and 1.5 h; (g) Boc_2O, DCM, 0 °C to rt, and 20 h; (h) *m*-CPBA, DCM, rt, and 3.5 h; (i) $EtNH_2$, H_2O, rt, and 72 h; (j) HCl, dioxane, rt, and 16 h; and (k) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, dioxane, 110 °C, and 24 h.

base, a reaction that proceeded with concomitant intramolecular cyclization. Copper-catalyzed coupling of **13** with iodoaryl **14** in the presence of N^1,N^2-dimethylethane-1,2-diamine and K_3PO_4 as bases provided the methylthio derivatives **15**, which were oxidized with *m*-CPBA and subsequently substituted with amines NHR_1R_2. The reduced derivative **23** was prepared in a seven-step sequence starting from aldehyde **18**. This involved reaction of **18** with N^1-methylethane-1,2-diamine (**12-1**), intramolecular reductive amination using $NaBH(OAc)_3$, protection of the amino group with Boc, oxidation of the methylthio group to the sulfone, substitution with ethylamine, Boc deprotection, and final Pd-catalyzed N-arylation of **21** with the bromoaryl compound **22** using the catalytic system $Pd_2(dba)_3$, Xantphos, and Cs_2CO_3.

Compounds **31**−**34** (Table 2) with a 3-methylamino-1-phenylpropoxy (MAP) moiety were prepared according to the sequence shown in Scheme 2. Key intermediates **27-1** (*n* = 0, X = I) were prepared by the Mitsunobu reaction of **24** with iodophenols **25** followed by the reaction with methylamine

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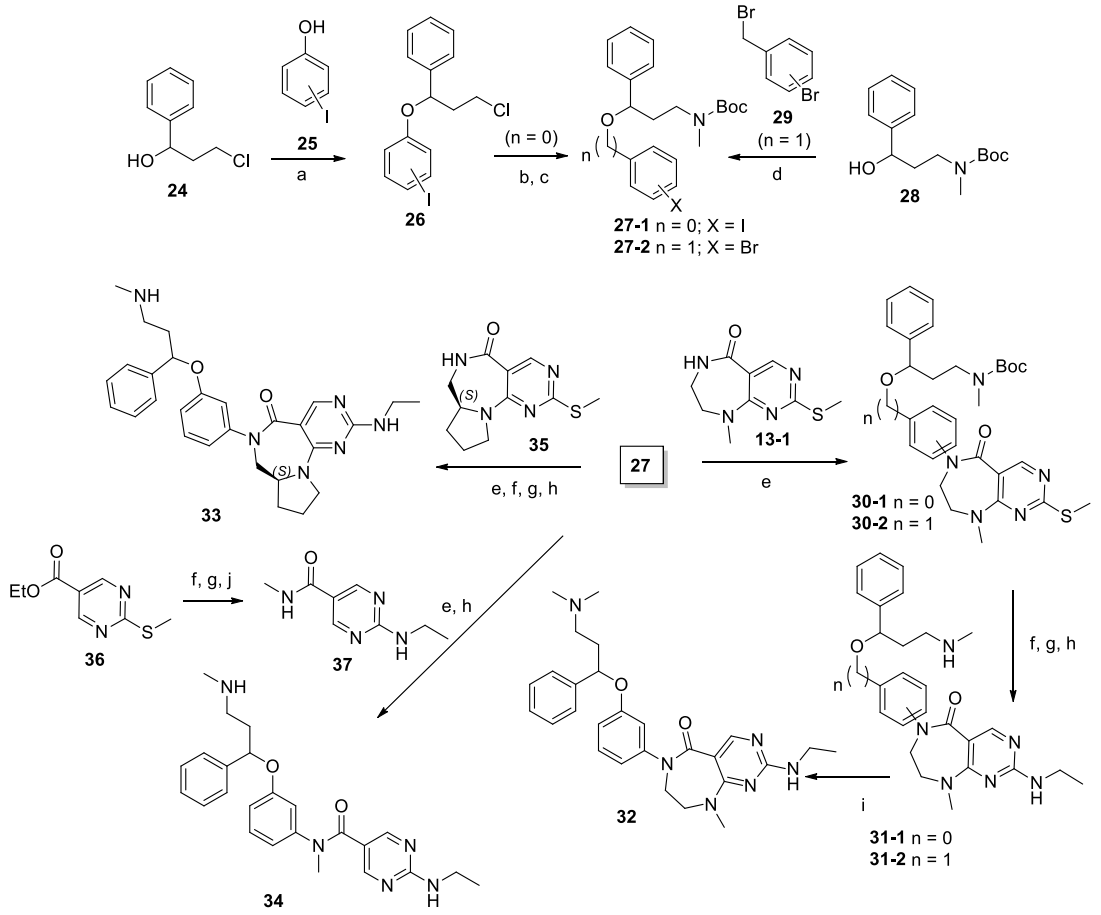
Scheme 2. Preparation of Compounds 31−34[a]



[a]Reagents and conditions: (a) PPh_3, DIAD, THF, rt, and 20 h; (b) methylamine, H_2O, EtOH, 130 °C, and 1 h; (c) Boc_2O, DCM, rt, and 2 h; (d) NaH, DMF, rt, and 3 h; (e) CuI, K_3PO_4, N^1,N^2-dimethylethane-1,2-diamine, dioxane, 110 °C, and 16 h; (f) *m*-CPBA, DCM, rt, and 1 h; (g) ethylamine, THF, rt, and 16 h; (h) HCl, dioxane, rt, and 45 min; (i) paraformaldehyde, $NaBH(OAc)_3$, DIPEA, AcOH, DCE, rt, and 70 h; and (j) methylamine, NaCN, EtOH, 100 °C, and 16 h.

and Boc protection of the amino group. Intermediates **27-2** (*n* = 1, X = Br) were obtained by alkylation of **28**[35] with bromobenzyl reagents **29** using NaH in DMF. Starting from compounds **27**, several final derivatives were obtained. Synthesis of compounds **31** involved copper-catalyzed N-arylation of **13-1** with **27**, oxidation of the thioether group to the sulfone, substitution with ethylamine, and final *N*-Boc deprotection, using the conditions described above. Compound **32**, with a dimethylamino substituent, was obtained in good yield by reductive amination of compound **31a** with paraformaldehyde. A similar methodology to that used for preparing compounds **31** was used for the synthesis of compound **33** using the tricyclic scaffold **35**, obtained as described in the literature.[23] In the case of **34**, the pyrimidine ethylamino substituent was already present in intermediate **37**, synthesized by an amidation reaction of **36** with methylamine. For the synthesis of pure enantiomers of compounds **31a** and **33**, commercial enantiopure intermediates **24** were used. No epimerization occurred in the Mitsunobu or alkylation reaction, and an enantiomeric excess of >96% was obtained. Throughout the manuscript, the suffix R or S after the number indicates that the compound is the corresponding pure enantiomer, while no suffix refers to a racemic derivative.

The 3-methylamino-1-(thiophen-2-yl)propoxy (MAT) derivatives described in Table 3 were prepared by a different methodology, outlined in Schemes 3, 4, 5. The Mitsunobu-based route of Scheme 2 was not successful when working with enantiopure derivative **38** since epimerization was observed in the aryl ether product. A better alternative was the O-arylation of **38** with 3-fluorobromobenzene **39** using NaH as the base, which afforded intermediate **40** in good yield and no decrease in the enantiomeric excess in relation to the starting material used (typically, values of >96% ee were obtained in the final compounds). The copper-catalyzed N-arylation reaction required protection of the methylamino group, and initially, *N*-Boc-protected derivatives **41-1** were prepared. However, in some cases, ether cleavage in the final deprotection step under acidic conditions was observed. Although this could be solved in some instances by using $ZnBr_2$ in DCM instead of HCl, the trimethylsilylethoxycarbonyl (Teoc)-protecting group was alternatively used. *N*-Teoc-protected intermediates **41-2** were easily prepared by treating **40** with 4-nitrophenyl (2-(trimethylsilyl)ethyl)carbonate. The N-arylation with **41-2** of different bicyclic scaffolds was performed as described above, and the final deprotection step, using CsF in DMF at 90 °C or under MW irradiation conditions, provided the final compounds. In the case of enantiopure derivatives, good enantiomeric excess was always obtained.

In this way, the pyrimido[4,5-*e*][1,4]diazepin-5-one derivatives (Scheme 3) were obtained. Reactions of **41-1** and **41-2**

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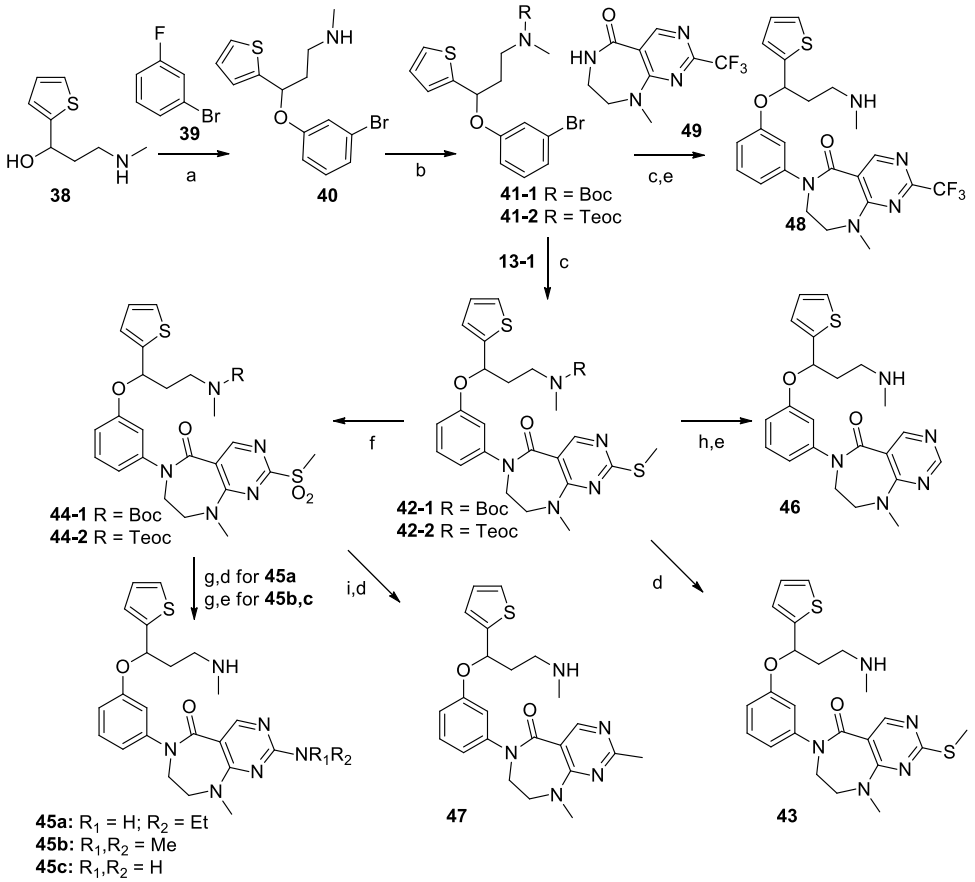
Scheme 3. Preparation of Pyrimido[4,5-*e*][1,4]diazepin-5-one Derivatives[a]



[a]Reagents and conditions: (a) NaH, DMA, 90 °C, and 3 h; (b) R = Boc: Boc_2O, DCM, rt, and 3 h; R = Teoc: 4-nitrophenyl-(2-(trimethylsilyl)ethyl)carbonate, DIPEA, DCM, rt, and 20 h; (c) CuI, K_3PO_4, N^1,N^2-dimethylethane-1,2-diamine, dioxane, 100 °C, and 20 h; (d) R = Boc: $ZnBr_2$, DCM, rt, 24 h; (e) R = Teoc: CsF, DMF, 90 °C, and 90 min; (f) *m*-CPBA, DCM, rt, and 1 h; (g) NHR_1R_2, THF, rt, and 20 h or MW at 100 °C and 30 min; (h) Pd/C, Et_3SiH, THF, 0 °C to rt, and 4 h; and (i) MeMgBr, THF, 0 °C, and 1 h.

with the methylthio-bicycle **13-1** provided compounds **42**. Compound **43** was prepared from **42-1** by deprotection of the Boc group using $ZnBr_2$ in DCM. Compound **42-1** was oxidized to the sulfone derivative **44-1**, which was reacted with ethylamine and deprotected with $ZnBr_2$ to provide compound **45a**, while the methyl derivative **47** resulted from methyl magnesium bromide attack to sulfone **44-1** and subsequent Boc deprotection. For the preparation of compounds **45b**, **45c**, **46**, and **48**, the Teoc-protecting group was preferred. The reaction of sulfone **44-2** with the corresponding amines, at rt or under MW irradiation at 100 °C, and deprotection with CsF in DMF provided derivatives **45b** and **45c**. The unsubstituted **46** was prepared by reduction of the thioether **42-2** with triethylsilane in the presence of Pd/C. The trifluoromethyl derivative **48** was prepared by the reaction of **41-2** with bicycle **49**, which in turn was obtained from ethyl 4-chloro-2-(trifluoromethyl)pyrimidine-5-carboxylate, using the conditions described above for the preparation of **13-1**.

The pyridodiazepin-5(2*H*)-one derivatives were synthesized (Scheme 4) from key intermediates **54** using in all cases the Teoc-protected derivative **41-2**. Compounds **54** were obtained from the two regioisomeric acid chlorides **50** by subsequent treatment with *N*-Boc-protected diamine **51** in the presence of Et_3N, deprotection of the Boc group, and intramolecular cyclization in the presence of CsF and Et_3N in DMF. Treatment of **54** with ethylamine under microwave irradiation gave the aminopyridyl scaffolds **55**, which were derivatized as described above to provide final compounds **56** and **57**. Halogenolysis of **54-2** to provide **58** was performed with triethylsilane and Pd/C in THF, and its final derivatization provided compound **59**. The Suzuki reaction of **54-2** with methylboronic acid provided scaffold **60**, which was further derivatized to compound **61**. The methoxy group was introduced with NaOMe in MeOH to give scaffold **62**, which was transformed to final compound **63**.

Final compounds **65**, **67**, **69**, and **71**, with alternative central scaffolds, were obtained (Scheme 5) by the reaction of **41-2** with the corresponding bicyclic intermediates **64**, **66**, **68**, and **70**, respectively, using the conditions described above. The pyrido derivatives **64** and **66** were prepared, in a similar way to that described above for the preparation of **13-1**, from the corresponding fluoropyridinyl esters. Scaffold **70** was commercially available, and **68** was obtained by reductive amination of commercially available **72** with paraformaldehyde.

SAR Studies. The biological activity of the compounds synthesized was evaluated in human $\alpha2\delta$-1- and $\alpha2\delta$-2-enriched membranes using [^{3}H]-gabapentin as the radioligand. Both targets were evaluated since in principle we aimed at finding selective agents for the $\alpha2\delta$-1 subunit. This was based on previous studies on the distribution and differential effects of the two subtypes,[36] suggesting that the analgesic effects of

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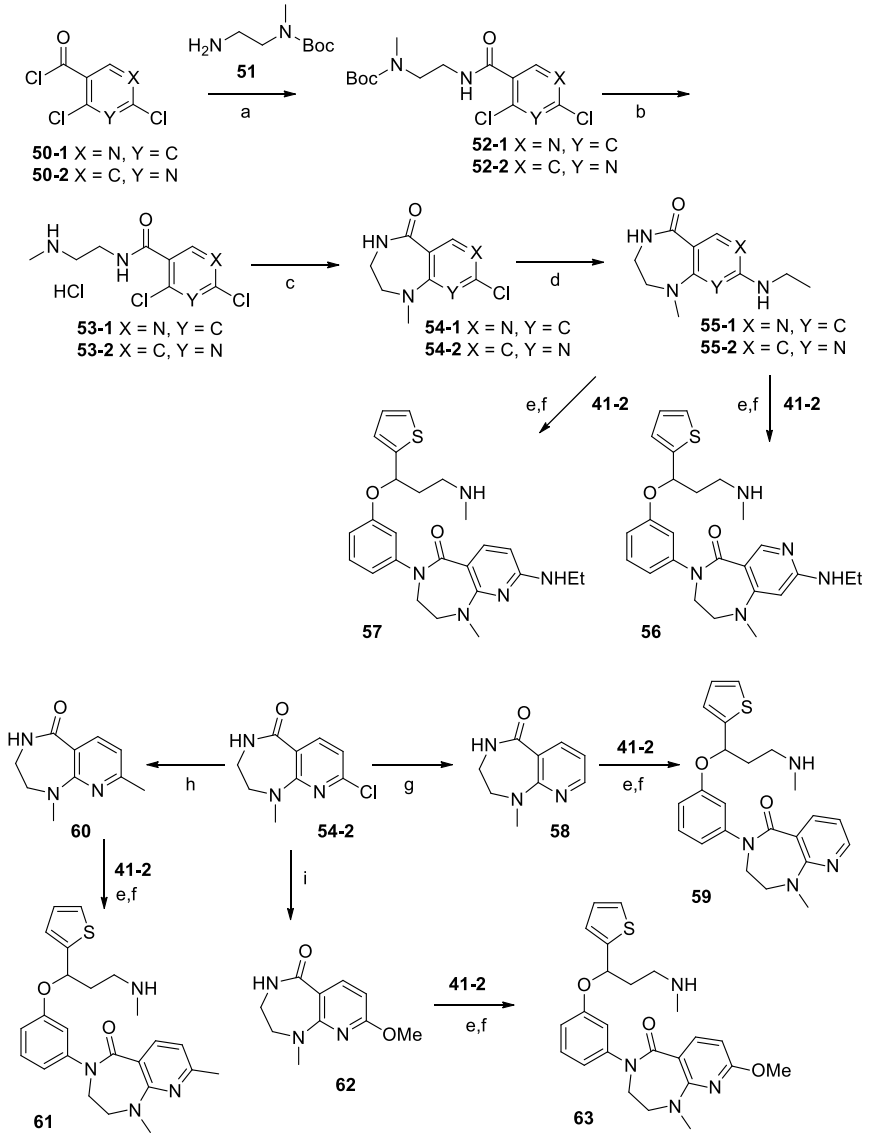
Scheme 4. Preparation of Pyridodiazepin-5(2*H*)-one Derivatives[a]



[a]Reagents and conditions: (a) Et_3N, THF, rt, and 2.5 h; (b) HCl, dioxane, rt, and 2 h; (c) CsF, Et_3N, DMF, 75 °C, and 16 h; (d) $EtNH_2$, H_2O, 135 °C, MW, and 2 h; (e) CuI, K_3PO_4, N^1,N^2-dimethylethane-1,2-diamine, dioxane, 100−130 °C, and 20 h; (f) CsF, DMF, 90 °C, and 90 min or MW at 90 °C and 90 min; (g) Et_3SiH, Pd/C, THF, rt, and 16 h; (h) $MeB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, dioxane, 130 °C, and 48 h; and (i) NaOMe, MeOH, 110 °C, MW, and 2 h.

compounds like pregabalin are due to the interaction with $\alpha2\delta$-1, while their major side effects such as sedation, dizziness, and ataxia may be caused by the interaction with $\alpha2\delta$-2. NET activity was evaluated by measuring the binding affinity to human NET-enriched membranes using [^{3}H]-nisoxetine as the radioligand and the functional activity in cells overexpressing NET and using 4-(4-(dimethylamino)styryl)-*N*-methylpyridinium iodide (ASP^+) as the fluorescent substrate. The results for the new compounds prepared are shown in Tables 1, 2, and 3, where the activities of reference compounds **1**, **6**, and **8** are also presented for comparison purposes.

For the design of the dual $Ca_v\alpha2\delta$-1 and NET ligands, we envisaged a merging approach from the scaffold represented by compound **6** and the NET pharmacophore common to compounds **7**−**10**. The SAR data around **6** were scarce since they were only described in a patent publication,[23] so we first focused on exploring the key features for $Ca_v\alpha2\delta$-1 binding and the most suitable positions, allowing substitution to attach the NET pharmacophore. The tricyclic scaffold of **6** was simplified by eliminating the fused pyrrolidine ring and keeping only a methyl group attached to the benzodiazepinone nitrogen (**I**, Figure 4). Maintaining an ethylamino group at the 2-position of the pyrimidine, we first explored the phenyl ring substitution. Several simple substituents were introduced in ortho-, meta-, and para-positions, and in general, they were poorly tolerated (results not shown), but a preferential trend for the meta-substitution was observed. The oxadiazolyl derivative in the meta-position was identified as the best substituent, and therefore, framework (**II**) was selected for further SAR studies (Table 1).

This study showed that the ethylamino group in position 2 was important for the activity since **16a** was the most potent compound, while shortening to methylamino (**16c**) provided a drop in affinity and the methylthio (**15-1**) and methylethyla-

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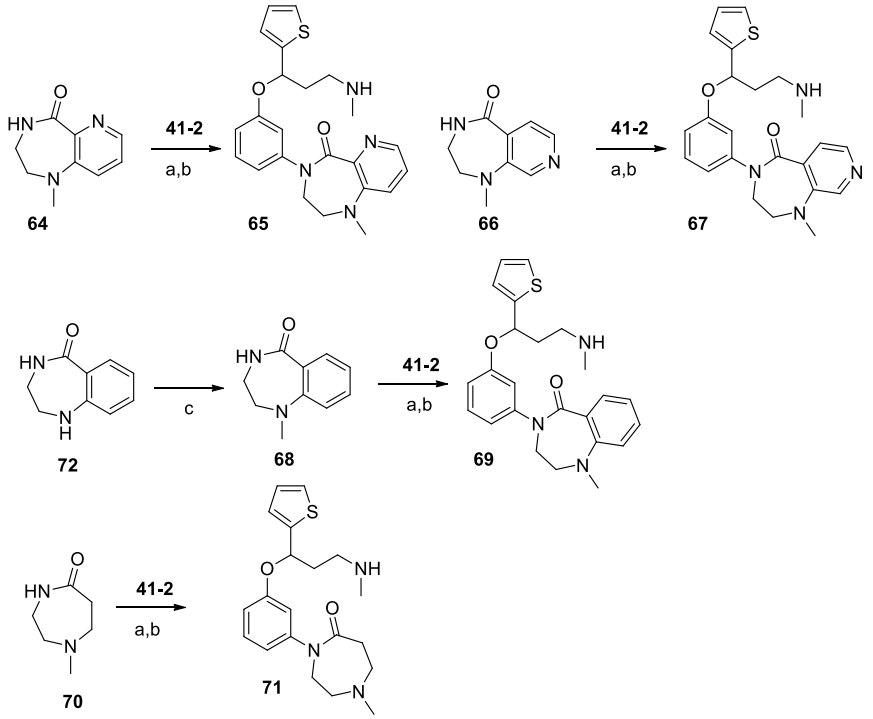
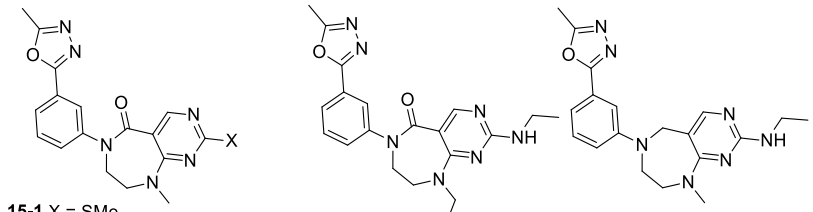
Scheme 5. Preparation of Compounds 65, 67, 69, and 71[a]



[a]Reagents and conditions: (a) CuI, K_3PO_4, N^1,N^2-dimethylethane-1,2-diamine, dioxane, 100 °C, and 20 h; (b) CsF, DMF, 90 °C, and 90 min; and (c) paraformaldehyde, $NaBH(OAc)_3$, DIPEA, AcOH, DCE, rt, 48 h.

Table 1. Initial Modifications of Compound 6



comp	$Ca_v\alpha2\delta$-1 (K_i, nM)[a]	$Ca_v\alpha2\delta$-2 (K_i, nM)[b]	NET (K_i, nM)[c]	NET (IC_{50}, nM)[d]
1	19 ± 12	99 ± 2[f]	>10,000[e]	>10,000[e]
6	11 ± 2	2148	>10,000[e]	>10,000[e]
8	>10,000[e]	>10,000[e]	39 ± 15	1.8 ± 1
15-1	>10,000[e]	>10,000[e]	>10,000[e]	>10,000[e]
16a	81 ± 15	2974	>10,000[e]	>10,000[e]
16b	>10,000[e]	NT[g]	>10,000[e]	>10,000[e]
16c	244 ± 97	>10,000[e]	>10,000[e]	>10,000[e]
16d	>10,000[e]	NT[g]	>10,000[e]	>10,000[e]
17	178 ± 72	NT[g]	>10,000[e]	>10,000[e]
23	1026 ± 999	70 ± 8[f]	>10,000[e]	>10,000[e]

[a]Binding affinity (K_i, nM) in human $\alpha2\delta$-1-enriched membranes from hamster tumor CHO-K1 cells (human Cav2.2/$\beta3$/$\alpha2\delta1$ calcium channel cell line, ChanTest) using [^{3}H]-gabapentin as the radioligand. Each value is the mean ± SD of two determinations. [b]Binding affinity (K_i, nM) in human $\alpha2\delta$-2-enriched membranes from human embryonic kidney HEK-293 cells using [^{3}H]-gabapentin as the radioligand. Each value is the mean ± SD of at least two determinations. [c]Binding affinity (K_i, nM) in human norepinephrine transporter (NET)-enriched membranes from human embryonic kidney HEK-293 cells using [^{3}H]-nisoxetine as the radioligand. Each value is the mean ± SD of at least two determinations. [d]Functional activity (IC_{50}, nM) in HEK-293 cells overexpressing human NET and using ASP^+ as the fluorescent substrate. Each value is the mean ± SD of two or three determinations. [e]Less than 50% inhibition at 10 μM. [f]% Inhibition at 10 μM. [g]Not tested.

mino (**16b**) derivatives were inactive. Elongating to methoxyethylamino (**16d**) and other substituents, including larger alkyl (butyl and pentyl), benzyl or phenyl groups provided a complete loss in affinity ($Ca_v\alpha2\delta1$ K_i > 10,000 nM). In the same way, enlarging position 9 was detrimental since the ethyl derivative **17** showed reduced affinity in relation to **16a**. Finally, the benzodiazepinone oxygen atom was proven to be necessary since the reduced derivative **23** was substantially less

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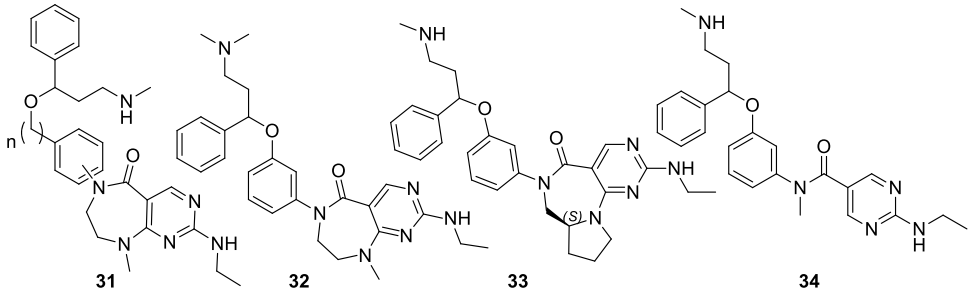
Table 2. Initial Exploration of the MAP Moiety Attachment to the $Ca_v\alpha2\delta$-1 Framework



31 32 33 34

comp	*n*, position	$Ca_v\alpha2\delta$-1 (K_i, nM)[a]	NET (K_i, nM)[b]	NET (IC_{50}, nM)[c]
31aR	0, meta	1417 ± 617	610 ± 242	203 ± 37
31aS	0, meta	2238 ± 990	29 ± 17	54 ± 2
31b	0, para	>10,000[d]	995 ± 581	716 ± 86
31c	0, ortho	3493 ± 17	>10,000[d]	>10,000[d]
31d	1, meta	706 ± 356	556 ± 85	184 ± 73
31e	1, para	6246 ± 2797	41 ± 7	7 ± 1
31f	1, ortho	>10,000[d]	>10,000[d]	>10,000[d]
32		2450 ± 2166	>10,000[e]	1862 ± 721
33R		187 ± 0	>10,000[d]	>10,000[d]
33S		336 ± 131	842 ± 70	1533 ± 300
34		>10,000[d]	>10,000[d]	>10,000[d]

[a]Binding affinity (K_i, nM) in human $\alpha2\delta$-1-enriched membranes from hamster tumor CHO-K1 cells (human Cav2.2/$\beta3/\alpha2\delta1$ calcium channel cell line, ChanTest) using [^{3}H]-gabapentin as the radioligand. Each value is the mean ± SD of at least two determinations. [b]Binding affinity (K_i, nM) in human norepinephrine transporter (NET)-enriched membranes from human embryonic kidney HEK-293 cells using [^{3}H]-nisoxetine as the radioligand. Each value is the mean ± SD of at least two determinations. [c]Functional activity (IC_{50}, nM) in HEK-293 cells overexpressing human NET and using ASP^+ as the fluorescent substrate. Each value is the mean ± SD of two or three determinations. [d]Less than 50% inhibition at 10 μM.

potent than **16a**. The properties of compound **16a** were considered to be a good starting point since it exhibited a good ADME profile, with adequate *in vitro* metabolic stability in human and rodent liver microsomes (intrinsic clearance below 5 μL/min/mg protein),[37] no potential for drug−drug interactions based on the low inhibition (<50% at 1 μM) of recombinant human cytochrome P450 isoforms (rhCYP 1A2, 2C9, 2C19, 2D6, and 3A4),[38] and IC_{50} above 10 μM in the human ether-a-go-go-related gene (hERG)[39] patch clamp assay, a well-known predictor of cardiac toxicity. Compound **16a** also exhibited selectivity for the $Ca_v\alpha2\delta$-2, for which it showed a K_i = 2974 nM. All the remaining new compounds reported here showed K_i values above 10 μM for the $Ca_v\alpha2\delta$-2, so these results are not reported in Tables 2 and 3.

The previous results indicated that substitution in positions 2 and 9 would not be tolerated, so we decided to explore the attachment of a NET pharmacophore onto the phenyl ring of the 2-ethylamino-9-methyl-6-phenyl-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*e*][1,4]diazepin-5-one scaffold. As the most representative NET framework, the 3-methylamino-1-phenylpropoxy (MAP) moiety was chosen, and the results of the initial exploration are provided in Table 2. Other shorter (3-methylamino-1-phenylethoxy) or longer (3-methylamino-1-phenylbutoxy) chains were also explored, but they provided weaker affinities (results not shown). We were pleased to see that the compound resulting from the MAP attachment in the meta-position, **31a**, provided dual activity, showing micromolar affinity for the $Ca_v\alpha2\delta$-1 and, interestingly, higher affinity for the NET. The *S* enantiomer showed nanomolar potency for NET, both in the binding and functional tests, and was clearly superior to the *R* isomer, a result consistent with the usual behavior of NET inhibitors.[40,41] On the contrary, pending the MAP moiety to the ortho- or para-position provided compounds **31b** and **31c**, with only residual affinities.

Next, we explored the introduction of flexibility through a methylene linker between the MAP moiety and the phenyl ring. The meta-derivative **31d** exhibited dual affinity but reduced NET affinity in relation to **31a**. The para-derivative **31e** was highly potent on the NET but poorly active on the $Ca_v\alpha2\delta$-1, and the ortho-derivative **31f** was again inactive. A dimethylamino group in place of the methylamino group on the MAP chain (compound **32**) proved to be clearly detrimental for NET inhibition. This result was consistent with previous findings in the monoamine transporter field, where dimethylation reduced NET affinity while maintaining or increasing SERT activity.[42] Attaching the MAP chain to the $Ca_v\alpha2\delta$-1 scaffold of **6** provided derivative **33**, with both enantiomers showing two-digit nanomolar affinity for the $Ca_v\alpha2\delta$-1 but poor activity at NET. Finally, the open derivative **34** was inactive for both targets. These results indicated that the proposed approach was indeed adequate to obtain dual derivatives, but that simultaneous improvement of activity in both targets would be challenging, as suggested by the comparison of **31aS** versus **33S**, where an increase in NET activity resulted in a decrease in $Ca_v\alpha2\delta$-1 activity and vice versa.

The phenyl group of the meta-MAP derivative **31a** was next changed to a 2-thiophenyl group to provide compound **45a**, where the *S* enantiomer was again the eutomer for NET activity, while both enantiomers kept similar $Ca_v\alpha2\delta$-1 affinities. The 3-methylamino-1-(thiophen-2-yl)propoxy (MAT) chain of **45aS** seemed to provide superior $Ca_v\alpha2\delta$-1 affinity versus its MAP counterpart, **31aS**, and was the framework selected to study the variation of the bicyclic core

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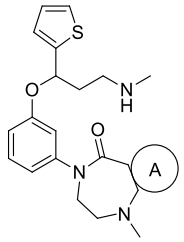
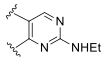
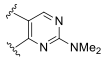
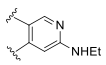
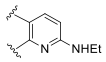
Table 3. Activity Data of MAT Derivatives Depicted in Schemes 3, 4, and 5



comp	A	Ca$_v$α2δ-1 (K$_i$, nM)[a]	NET (K$_i$, nM)[b]	NET (IC$_{50}$, nM)[c]
43S		815 ± 839	4 ± 7	0.6 ± 0.2
45aR		492 ± 259	>1000[d]	601 ± 440
45aS	"	480 ± 87	182 ± 100	46 ± 46
45b		923 ± 67	356 ± 87	220 ± 41
45cS		454 ± 233	59 ± 26	7 ± 5
46S		359 ± 55	77 ± 67	9 ± 0
47S		968 ± 322	60 ± 47	10 ± 2
48		3316 ± 15	69 ± 27	58 ± 13
56S		263 ± 57	82 ± 133	210 ± 102
57S		755 ± 365	75 ± 22	41 ± 6
59S		112 ± 8	383 ± 158	67 ± 24
61S		151 ± 13	305 ± 84	109 ± 18
63S		367 ± 117	105 ± 3	3 ± 4
65		1246 ± 674	>1000[d]	213 ± 35
67		208 ± 35	341 ± 67	341 ± 67
69		833 ± 733	>1000[d]	815 ± 462
71R	H_2,H_2	606 ± 119	>1000[d]	>1000[d]
71S	H_2,H_2	2797 ± 20	382 ± 66	97 ± 28

[a]See footnotes of Table 2. [b]See footnotes of Table 2. [c]See footnotes of Table 2. [d]Less than 50% inhibition at 1 μM.

(Table 3). It should be noted that in many cases, the racemic compounds were first prepared and for the most active derivatives, the *S* enantiomers (which were consistently the eutomers for NET, following the trends of **31aR/S** and **45aR/S**) were then synthesized. For the sake of the length of the paper, we report only the *S* enantiomers when available.

Surprisingly, intermediate **43S** showed a very high NET activity both in the binding and functional assay as well as submicromolar Ca$_v$α2δ-1 activity, a result unexpected in view of the low Ca$_v$α2δ-1 affinity of **15-1**. This result indicated that the 2-position, where the ethylamino group was identified as key for Ca$_v$α2δ-1 activity in the first study (Table 1), could be further explored. The dimethylamino derivative **45b** showed a worsening in all activities, and the amino **45cS** and unsubstituted **46S** were again fairly potent in both targets. The methyl (**47S**) and trifluoromethyl (**48**) derivatives showed an impairment at Ca$_v$α2δ-1.

Deletion of one nitrogen atom of the pyrimido bicycle provided the pyrido derivatives **56S** and **57S**, with dual affinities comparable to those of **45aS**. Many analogues of both pyridine isomers were prepared, and only a handful of the most active compounds (**59S**, **61S**, and **63S**) are reported in Table 3. The other two pyrido isomers **65** and **67** showed a decreased affinity at NET, similar to the benzo derivative **69**. The monocyclic diazepinone **71** showed a substantial decrease in affinity, with NET activity retained in the *S* enantiomer and Ca$_v$α2δ-1 activity mainly in the *R* enantiomer.

Several of the compounds reported in Tables 2 and 3 show quite a balanced dual profile, but in general, the compounds are more potent in NET than in the Ca$_v$α2δ-1, with several compounds, such as **31aS** and **43S**, with outstanding NET results. These findings were surprising since we were expecting the opposite as many of the NET inhibitors described in the literature correspond to small ligands circumscribed to structures related to those depicted in Figure 2. When analyzing the NET structural information published by the group of Gouaux, first for LeuT, bacterial homolog of biogenic amine transporters (BATs), engineered to achieve human BAT-like pharmacology,[42] and later on for the human SERT,[43] we guessed that a cavity located near the primary binding site, corresponding to a low affinity or allosteric site in the homologous proteins, might allow the accommodation of larger derivatives, such as **31aS** and **43S**.

To check this hypothesis and get more knowledge about the key ligand−receptor interactions, which might help in a later optimization program, we built a NET homology model using as a template the six crystallized SERT structures (details given in the Experimental Section) and docked into it compound **31aS**. We observed that the MAP moiety was positioned in the expected primary binding site, orienting the bicyclic diazepinone framework in a contiguous site in the extracellular vestibule. We embedded the resulting docked structure in an explicit lipid bilayer, solvated it, and ran a 1 ms MD simulation. The main interactions observed in the resulting trajectory are schematically drawn in Figure 5. The MAP amine group interacts with the carboxylate of the conserved Asp75 and with Phe72, with which it establishes cation−π interactions. Ser318 and Ser419 on each side of the ligand basic amine interact with it directly (Ser318) or through water bridges (Ser419), defining together with Asp75 and Phe72 what has been previously defined[43] as subsite A. Subsite B is occupied by the phenyl group of the MAP moiety engaging in π−π stacking interactions with Phe323, mostly edge to face, and in

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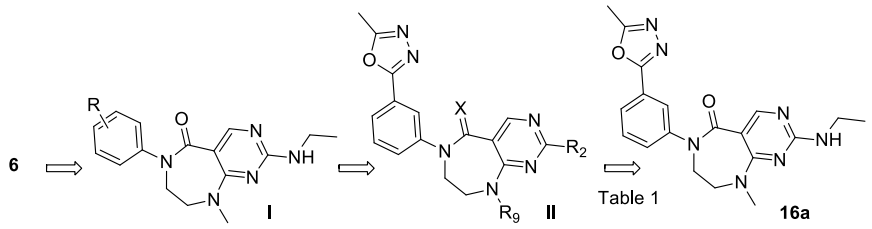

Figure 4. Modification sequence from **6** to **16a**.

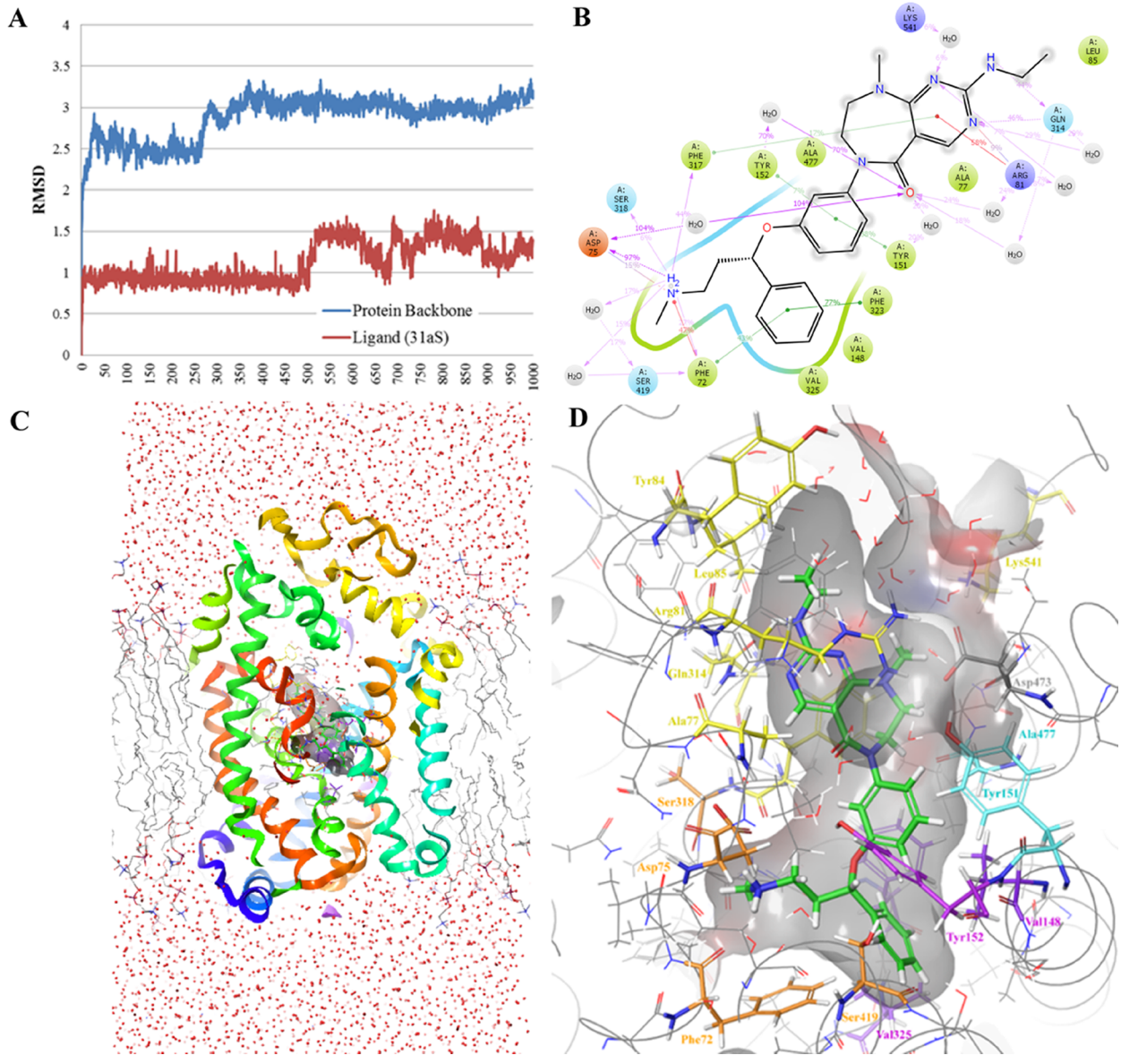

Figure 5. Docking of compound **31aS** in the NET homology model generated from the SERT structures l5I6X, 5I6Z, 5I71, 5I73, 5I74, and 5I75. (A) RMSD of the protein and the docked ligand in the 1 μs molecular dynamics simulations. (B) 2D representation of the interactions of **31aS** in the active site of NET. (C) Image of NET embedded in the lipid bilayer and solvated. (D) Compound **31aS** in the NET binding site; the aminoacids belonging to the different subsites have been colored in orange for subsite A, purple for B, cyan for C, and yellow for the residues belonging to the allosteric site.

hydrophobic interactions with Val148, Val325, and Tyr152. These residues together with Phe323 start defining subsite C by π stacking with the phenyl that constitutes the intersection of the original $Ca_v\alpha2\delta$-1 scaffold with the NET pharmacophore. This phenyl ring additionally establishes hydrophobic interactions with Ala477 and Val148 and forms an edge-to-face aromatic interaction with Tyr151. Subsite C connects the primary binding site with a site in the extracellular vestibule, which is able to harbor the $Ca_v\alpha2\delta$-1 pharmacophore. It is lined by transmembrane helices 1, 6, and 10 and located halfway between the sites found in LeuT and SERT. The pyrimidodiazepinone ring system is stabilized by hydrophobic

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interactions with Ala77, Phe317, and Ala477 and by ionic interactions of Arg81 with the nitrogen atoms in positions 1 and 3 and of the 2-ethylamino group. Additional hydrogen bonding is established with Gln314 and one or both the 2-ethylamino group and the nitrogen in position 3 of the pyrimidine ring. This H bond interaction between the ligand and the amide of Gln314 may explain the worsening in NET affinity of the dimethylamino derivative **45b**, and altogether, the mentioned polar interactions in this subsite explain the convenience of polar substitution in the bicycle and the complete loss of NET affinity of the benzodiazepinone **69**. Further insight into the intriguing binding of this compound series in the NET will be reported in due course.

Besides exhibiting promising dual affinities, several compounds among those reported in Table 3 showed a good ADMET profile. For example, compounds **45cS** and **59S** showed a balanced dual profile accompanied by a good physicochemical profile (MW: 438.5 and 422.5, respectively; cLogP: 2.4 and 3.2, respectively; and kinetic solubility above 10 μM in both cases), an adequate metabolic stability in human, mouse, and rat liver microsomes (intrinsic clearance below 5 μL/min/mg protein), no potential for drug−drug interactions based on the low inhibition (<50% at 1 μM) of recombinant human cytochrome P450 isoforms (rhCYP 1A2, 2C9, 2C19, 2D6, and 3A4), and IC_{50} above 10 μM in the human ether-a-go-go-related gene (hERG) patch clamp assay. These results prompted the initiation of a lead optimization program toward the finding of a preclinical candidate dual compound for the treatment of pain, which identification will be reported soon.

CONCLUSIONS

In summary, the synthesis and pharmacological activity of a new series of bicyclic diazepinones with dual activity toward the $Ca_v\alpha2\delta$-1 and the NET have been studied. A non-aminoacidic $Ca_v\alpha2\delta$-1 scaffold described in the literature was simplified, and its different positions were explored in order to identify $Ca_v\alpha2\delta$-1 key binding features and the best positions for the attachment of NET pharmacophores. Among the patterns explored, substitution with the 3-methylamino-1-phenylpropoxy (MAP) and 3-methylamino-1-thiophenylpropoxy (MAT) moieties in the meta-position of the phenyl ring of the 2-ethylamino-9-methyl-6-phenyl-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*e*][1,4]diazepin-5-one scaffold was the most interesting. The dual compounds showed excellent NET functionality despite being substantially larger than prototypic NET inhibitors described in the literature. The existence of a cavity located near the NET primary binding site, which might allow the accommodation of larger derivatives, can explain this fact.

Changing the central scaffold by other bicycles provided balanced dual compounds that also exhibited good ADMET properties and were the basis for a lead optimization program, which allowed the identification of a suitable preclinical candidate for the treatment of pain that will be published soon.

EXPERIMENTAL SECTION

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Reference compounds were obtained as follows: pregabalin (**1**)[44] and compound **6**[23] were prepared following the routes described in the literature, and reboxetine (**8**) was acquired from Waterstonetech (WS50505). Flash chromatography was performed on a Teledyne Isco CombiFlash RF system with disposable columns. NMR spectra were recorded on a Bruker Fourier 300, Bruker Avance I 400, or Bruker Avance I 500, operating at frequencies of 300, 400, and 500 MHz for ^{1}H and 75, 100, and 125 MHz for ^{13}C, respectively. Chemical shifts (δ) are in parts per million. Accurate mass measurements were carried out using a 6540 UHD high-resolution mass spectrometer QTOF system and obtained by electron spray ionization (ESI) in positive mode. Data were acquired and processed using MassHunter software. Commercially available reagents and solvents (HPLC grade) were used without further purification for all the analytical tests. Analytical HPLC purity data for all final compounds were obtained on an Agilent HP 1200-MS 6120 system using method 1: Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm), gradient 5−95% B (A = H_2O (0.05% TFA), B = acetonitrile) over 7 min, then isocratic 95% B for 5 min, injection volume: 1 μL, and flow: 1 mL/min. UV spectra were recorded at 254 nm using an Agilent HP 1200 VWD detector. Data were integrated and reported using Agilent ChemStation software. All compounds showed purity higher than 95% as determined by this method. Chiral SFC analysis of intermediates for ee determination was performed on a Waters ACQUITY UPC^2 system using method 2: CHIRALPAK IB column (4.6 × 100 mm, 3 μM), IPA/CO_2 5:95, and 230 nm. Chiral analytical HPLC of final compounds for ee determination was performed in an Agilent 1260 system. The coated or immobilized polysaccharide columns were acquired from Chiral Technologies Europe. Three methods were used: method 3: CHIRALCEL AS-H column (4.6 × 250 mm, 5 μm), isocratic *n*-heptane/ethanol + 0.33% DEA 80:20, injection volume: 5 μL, and flow: 0.8 mL/min; method 4: CHIRALCEL AS-H column (4.6 × 250 mm, 5 μm), isocratic *n*-heptane/ethanol + 0.33% DEA 70:30, injection volume: 5 μL, and flow: 0.8 mL/min; and method 5: CHIRALPAK AD-H column (4.6 × 250 mm, 5 μm), isocratic *n*-heptane/*iso*-propanol + 0.33% DEA 70:30, injection volume: 5 μL, and flow: 0.8 mL/min. All compounds active in biological assays were electronically filtered for structural attributes common to pan-assay interference compounds (PAINS) and were found to be negative.[45]

Determination of Physicochemical Properties. pK_a was calculated using ACDLabs 9.0.3 and cLog*P* using ChemDraw Ultra 10.0.3. Kinetic solubility was measured by HPLC after centrifugation of a sample obtained by adding phosphate buffer at pH = 7.4 (1 mL) to 10 μL of a 10 mM solution of the test compound in DMSO and stirring for 4 h.

General Procedures for the preparation of compound. *s9-Methyl-2-(methylthio)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]-diazepin-5-one (**13-1**).* A solution of potassium *tert*-butoxide (1.0 g, 8.42 mmol) in THF (0.2% water content, 25 mL) was stirred under air at rt for 10 min. Then, a solution of ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate (**11**, 1.0 g, 4.21 mmol) and N^1-methylethane-1,2-diamine (**12-1**, 329 mg, 4.21 mmol) in THF (0.2% water content, 18 mL) was added dropwise and the mixture was stirred at rt for 20 h. Additional amounts of potassium *tert*-butoxide were added after 4 h (4.21 mmol) and 16 h (6.31 mmol) to complete the reaction. The reaction mixture was concentrated under vacuum, DCM was added, and the solution was washed successively with water, NH_4Cl saturated solution, and brine. The organic phase was concentrated under vacuum to afford **13-1** (388 mg, 41% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 8.65 (s, 1H), 4.86 (s, 3H), 3.73 (m, 2H), 3.51 (m, 2H), 2.55 (s, 3H).

*9-Methyl-6-(3-(5-methyl-1,3,4-oxadiazol-2-yl)phenyl)-2-(methylthio)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]diazepin-5-one (**15-1**).* To a solution of compound **13-1** (71 mg, 0.31 mmol) and 2-(3-iodophenyl)-5-methyl-1,3,4-oxadiazole (**14**)[46] (145 mg, 0.50 mmol) in dioxane (3 mL), CuI (30 mg, 0.15 mmol), K_3PO_4 (134 mg, 0.63 mmol), and N^1,N^2-dimethylethane-1,2-diamine (17 μL, 0.15 mmol) were added, and the mixture was heated at 100 °C under an Ar atmosphere for 16 h. The reaction mixture was cooled to rt and filtered through a pad of Celite, and the solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient hexane to 100% acetone, afforded **15-1** (15 mg, 13% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.82 (bs, 1H), 7.97 (t, J = 1.5 Hz, 1H), 7.92

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(dt, J = 1.5, 7.7 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.53−7.51 (m, 1H), 4.09−4.07 (m, 2H), 3.87−3.85 (m, 2H), 3.35 (s, 3H), 2.64 (s, 3H), 2.59 (s, 3H). HPLC, method 1: RT, 5.03 min; purity, 98.0%. HRMS calcd for $C_{18}H_{19}N_6O_2S$, 383.1285 $[M + H]^+$; found, 383.1275 $[M + H]^+$.

*2-(Ethylamino)-9-methyl-6-(3-(5-methyl-1,3,4-oxadiazol-2-yl)-phenyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]diazepin-5-one (**16a**).* To a solution of **15-1** (66 mg, 0.17 mmol) in DCM (1.5 mL), *m*-CPBA (55 mg, 0.22 mmol) was added, and the mixture was stirred at rt for 1 h. $NaHCO_3$ saturated solution was added, and the mixture was extracted with DCM. The organic layer was washed with brine and dried with Na_2SO_4, and the solvent was removed under vacuum. The crude product was dissolved in THF (1 mL), ethylamine (0.43 mL of a 2 M solution in THF, 0.86 mmol) was added, and the mixture was stirred at rt for 16 h. The crude residue was concentrated under vacuum and purified by flash chromatography, silica gel, gradient hexane to 100% acetone, to afford compound **16a** (40 mg, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (bs, 1H), 7.95 (t, J = 1.5 Hz, 1H), 7.90 (dt, J = 1.7, 7.0 Hz, 1H), 7.47−7.54 (m, 2H), 5.78 (bs, 1H), 4.04−4.02 (m, 2H), 3.78−3.75 (m, 2H), 3.47 (m, 2H), 3.23 (s, 3H), 2.60 (s, 3H), 1.24 (t, J = 7.2 Hz, 3H). HPLC, method 1: RT, 4.88 min; purity, 98.4%. HRMS calcd for $C_{19}H_{22}N_7O_2$, 380.1829 $[M + H]^+$; found, 380.1831$[M + H]^+$.

*4-((2-Aminoethyl)(methyl)amino)-2-(methylthio)pyrimidine-5-carbaldehyde (**19**).* To a solution of 4-chloro-2-(methylthio)-pyrimidine-5-carbaldehyde (**18**, 712 mg, 3.77 mmol) in acetonitrile (40 mL) at 0 °C, Et_3N (2.47 mL, 17.74 mmol) and N^1-methylethane-1,2-diamine (**12-1**, 0.42 mL, 4.91 mmol) were added, and the mixture was stirred at rt for 20 h. $NaHCO_3$ saturated solution was added and extracted with EtOAc. The organic phase was concentrated under vacuum, and the residue was purified by flash chromatography, silica gel, gradient DCM to 30% MeOH, to afford compound **19** (627 mg, 73% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.56 (s, 3H), 3.34 (s, 3H), 3.51 (m, 2H), 4.04 (m, 2H), 8.06 (m, 1H), 8.16 (s, 1H).

*tert-Butyl 9-Methyl-2-(methylthio)-5,7,8,9-tetrahydro-6H-pyrimido[4,5-e][1,4]diazepine-6-carboxylate (**20**).* To a solution of compound **19** (210 mg, 0.92 mmol) in DCE (38 mL), $NaBH(OAc)_3$ (393 mg, 1.85 mmol) was added, and the reaction mixture was stirred at rt for 1.5 h. $NaHCO_3$ saturated solution was added and extracted with DCM. The organic phase was concentrated under vacuum, and the residue was purified by flash chromatography, silica gel, gradient DCM to 20% MeOH, to afford 9-methyl-2-(methylthio)-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*e*][1,4]diazepine (152 mg, 78% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (s, 1H), 4.03 (m, 2H), 3.52 (m, 2H), 3.34 (s, 3H), 2.55 (s, 3H).

To a solution of the previous compound (145 mg, 0.69 mmol) in DCM (6.5 mL) at 0 °C, di-*tert*-butyldicarbonate (150 mg, 0.69 mmol) was added, and the mixture was stirred at rt for 20 h. $NaHCO_3$ saturated solution was added, extracted with DCM, and concentrated under vacuum to afford compound **20** that was used in the next step without further purification (214 mg, quant yield). ^{1}H NMR (300 MHz, CD_3OD): δ rotamers 7.75 and 7.69 (s, 1H), 4.51 and 4.45 (s, 2H), 3.79 (m, 4H), 3.20 (s, 3H), 2.49 (s, 3H), 1.45 and 1.38 (s, 9H).

*N-Ethyl-9-methyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]-diazepin-2-amine (**21**).* To a solution of compound **20** (227 mg, 0.73 mmol) in DCM (4 mL), *m*-CPBA (252 mg, 1.46 mmol) was added, and the mixture was stirred at rt for 3.5 h. $NaHCO_3$ saturated solution was added, and the mixture was extracted with DCM. The organic layer was dried with $MgSO_4$, and the solvent was removed under vacuum. The crude residue was treated with ethylamine (70% solution in water, 2.1 mL, 26.10 mmol), and the mixture was stirred at rt for 72 h. The reaction mixture was concentrated under vacuum and purified by flash chromatography, silica gel, gradient DCM to 20% MeOH, to afford *tert*-butyl 2-(ethylamino)-9-methyl-5,7,8,9-tetrahydro-6*H*-pyrimido[4,5-*e*][1,4]diazepine-6-carboxylate (94 mg, 47% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (t, J = 7.2 Hz, 3H), 1.43 (s, 9H), 3.10 (s, 3H), 3.40 (m, 2H), 3.57 (m, 2H), 3.73 (m, 2H), 4.29 and 4.41 (s, 2H), 5.06 (bs, 1H), 7.59 and 7.69 (s, 1H).

To a solution of the previous compound (94 mg, 0.30 mmol) in dioxane (0.3 mL), HCl (4 M solution in dioxane, 1.0 mL, 4.28 mmol) was added, and the mixture was stirred at rt for 16 h. The reaction mixture was concentrated under vacuum, 10% Na_2CO_3 aqueous solution was added and extracted with DCM, and the organic phase was concentrated to dryness to afford compound **21** (61 mg, 96% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 7.57 (s, 1H), 3.70 (s, 2H), 3.47 (m, 2H), 3.34 (q, J = 7 Hz, 2H), 3.11 (m, 2H), 3.08 (s, 3H), 1.18 (t, J = 7 Hz, 3H).

*N-Ethyl-9-methyl-6-(3-(5-methyl-1,3,4-oxadiazol-2-yl)phenyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]diazepin-2-amine (**23**).* A mixture of $Pd_2(dba)_3$ (5 mg, 0.0053 mmol), Xantphos (5 mg, 0.012 mmol), compound **21** (30 mg, 0.14 mmol), 2-(3-bromophenyl)-5-methyl-1,3,4-oxadiazole (**22**, 25 mg, 0.10 mmol), and Cs_2CO_3 (48 mg, 0.14 mmol) in dry dioxane (0.5 mL) under argon was heated at 110 °C in a sealed tube for 24 h. The reaction mixture was cooled to rt and filtered through a pad of Celite, and the filtrate was concentrated under vacuum. Purification by flash chromatography, silica gel, gradient hexane to 100% acetone, afforded compound **23** (6 mg, 15% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (bs, 1H), 7.39 (m, 1H), 7.34−7.29 (m, 2H), 6.88−6.85 (m, 1H), 5.14 (bs, 1H), 4.48 (s, 2H), 3.80−3.76 (m, 4H), 3.42−3.35 (m, 2H), 3.12 (s, 3H), 2.62 (s, 3H), 1.20 (t, J = 7.2 Hz, 3H). HPLC, method 1: RT, 5.51 min; purity, 95.1%. HRMS calcd for $C_{19}H_{24}N_7O$, 366.2037 $[M + H]^+$; found, 366.2031 $[M + H]^+$.

*(R)-1-(3-Chloro-1-phenylpropoxy)-3-iodobenzene (**26R-meta**).* To a solution of (*S*)-3-chloro-1-phenylpropan-1-ol (**24S**, 400 mg, 2.34 mmol) in THF (11 mL), 3-iodophenol (774 mg, 3.52 mmol) and PPh_3 (922 mg, 3.52 mmol) were added. The solution was cooled at 0 °C, DIAD (0.692 mL, 3.52 mmol) was added dropwise with rapid stirring, and the reaction mixture was stirred at rt for 20 h. The solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient hexane to 100% ethyl acetate, afforded compound **26R-meta** (779 mg, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41−7.36 (m, 4H), 7.32 (m, 1H), 7.29 (m, 1H), 7.25 (m, 1H), 6.91 (t, J = 8.5 Hz, 1H), 6.82 (m, 1H), 5.37 (m, 1H), 3.84−3.77 (m, 1H), 3.65−3.58 (m, 1H), 2.53−2.43 (m, 1H), 2.28−2.18 (m, 1H).

*tert-Butyl (R)-(3-(3-Iodophenoxy)-3-phenylpropyl)(methyl)-carbamate (**27-1R-meta**).* To a solution of **26R-meta** (779 mg, 2.09 mmol) in EtOH (2.3 mL), methylamine (40% solution in water, 5.4 mL, 62.7 mmol) was added, and the mixture was heated at 130 °C in a sealed tube for 1 h. The reaction mixture was cooled at rt, water was added, the mixture was extracted with DCM, and the organic phases were concentrated under vacuum. The crude product was dissolved in DCM (17 mL), di-*tert*-butyldicarbonate (502 mg, 2.30 mmol) was added at 0 °C, and the reaction mixture was stirred at rt for 2 h. Water was added, and the mixture was extracted with DCM and washed with $NaHCO_3$ saturated solution and brine. The solvent was removed under vacuum, and the residue was purified by flash chromatography, silica gel, gradient cyclohexane to 100% EtOAc, to afford compound **27-1R-meta** (838 mg, 86% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41−7.26 (m, 5H), 7.25 (m, 1H), 7.21 (m, 1H), 6.88 (t, J = 8.5 Hz, 1H), 6.77 (m, 1H), 5.10 (m, 1H), 3.56−3.26 (m, 2H), 2.87 (s, 3H), 2.28−2.01 (m, 2H), 1.52−1.33 (bs, 9H). Chiral SFC analysis, method 2: RT, 2.63 min; 96% ee.

*tert-Butyl (3-((3-Bromobenzyl)oxy)-3-phenylpropyl)(methyl)-carbamate (**27-2-meta**).* To a solution of *tert*-butyl (3-hydroxy-3-phenylpropyl)(methyl)carbamate (**28**, 115 mg, 0.43 mmol) in DMF (6 mL) cooled at 0 °C, NaH (35 mg, 60% in mineral oil, 0.86 mmol) was added, and the solution was stirred at rt for 30 min. Then, a solution of 1-bromo-3-(bromomethyl)benzene (162 mg, 0.65 mmol) in DMF (2 mL) was added at 0 °C and the mixture was stirred at rt for 3 h. Water was added, and the mixture was extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered, and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, afforded compound **27-2-meta** (159 mg, 80% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48 (s, 1H), 7.44−7.37 (m, 3H), 7.37−7.30 (m, 3H), 7.26−7.19 (m, 2H), 4.40 (d, J = 12.1 Hz, 1H), 4.34 (m, 1H), 4.25 (d, J = 12.1 Hz, 1H), 3.41−3.22 (m, 2H), 2.85 (s, 3H), 2.07 (m, 1H), 1.94 (m, 1H), 1.45 (s, 9H).

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*tert-Butyl (R)-Methyl(3-(3-(9-methyl-2-(methylthio)-5-oxo-5,7,8,9-tetrahydro-6H-pyrimido[4,5-e][1,4]diazepin-6-yl)phenoxy)-3-phenylpropyl)carbamate (**30aR**).* To a solution of **13-1** (150 mg, 0.67 mmol) and **27-1R-meta** (313 mg, 0.67 mmol) in dioxane (10 mL), CuI (38 mg, 0.20 mmol), K_3PO_4 (284 mg, 1.33 mmol), and N^1,N^2-dimethylethane-1,2-diamine (17 mg, 0.20 mmol) were added, and the mixture was heated at 110 °C under an Ar atmosphere for 16 h. The reaction mixture was cooled to rt, and the solvent was removed under vacuum to give a crude product that was purified by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, to afford compound **30aR** (267 mg, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1H), 7.35 (m, 4H), 7.28 (m, 1H), 7.20 (m, 1H), 6.82 (m, 2H), 6.72 (m, 1H), 5.13 (m, 1H), 3.90 (m, 2H), 3.71 (m, 2H), 3.41 (m, 2H), 3.28 (s, 3H), 2.86 (s, 3H), 2.57 (s, 3H), 2.14 (m,2 H), 1.42 (s, 9H).

*(R)-2-(Ethylamino)-9-methyl-6-(3-(3-(methylamino)-1-phenylpropoxy)phenyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]-diazepin-5-one (**31aR**).* To a solution of **30aR** (187 mg, 0.33 mmol) in DCM (6 mL), *m*-CPBA (123 mg, 0.49 mmol) was added, and the mixture was stirred at rt for 1 h. $NaHCO_3$ saturated solution was added, and the mixture was extracted with DCM. The organic layer was washed with brine and dried with Na_2SO_4, and the solvent was removed under vacuum. The crude product was dissolved in THF (6 mL), ethylamine (1.66 mL of a 2 M solution in THF, 3.32 mmol) was added, and the mixture was stirred at rt for 16 h. The crude residue was concentrated under vacuum and purified by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, to afford *tert*-butyl (*R*)-(3-(3-(2-(ethylamino)-9-methyl-5-oxo-5,7,8,9-tetrahydro-6*H*-pyrimido[4,5-*e*][1,4]diazepin-6-yl)phenoxy)-3-phenylpropyl)(methyl)carbamate (175 mg, 94% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71 (s, 1H), 7.35 (m, 4H), 7.26 (m, 1H), 7.17 (m, 1H), 6.83 (m, 2H), 6.68 (m, 1H), 5.11 (m, 1H), 3.87 (m, 2H), 3.63 (m, 2H), 3.48 (m, 2H), 3.40 (m, 2H), 3.20 (s, 3H), 2.85 (s, 3H), 2.16 (m, 1H), 2.09 (m, 1H), 1.42 (s, 9H), 1.25 (t, J = 7 Hz, 3H).

To a solution of the previous compound (128 mg, 0.22 mmol) in dioxane (0.4 mL), HCl (4 M in dioxane, 0.800 mL, 3.20 mmol) was added, and the mixture was stirred at rt for 45 min. The reaction mixture was concentrated to dryness under vacuum. To obtain the free base, DCM was added and washed with Na_2CO_3 (10% aqueous solution), and the aqueous phase was extracted with DCM. The combined organic layers were concentrated under vacuum to afford **31aR** (101 mg, 89% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.58 (bs, 1H), 7.48−7.46 (m, 2H), 7.42−7.38 (m, 2H), 7.34−7.26 (m, 2H), 6.92−6.91 (m, 1H), 6.89−6.85 (m, 2H), 5.42 (dd, J = 4.8, 8.0 Hz, 1H), 3.94−3.92 (m, 2H), 3.77−3.76 (m, 2H), 3.52−3.47 (m, 2H), 3.29 (s, 3H), 2.85−2.73 (m, 2H), 2.44 (s, 3H), 2.31−2.20 (m, 1H), 2.14−2.06 (m, 1H), 1.29 (t, J = 7.2 Hz, 3H). HPLC, method 1: RT, 4.95 min; purity, 97.2%. HRMS calcd for $C_{26}H_{32}N_6O_2$, 461.266 $[M + H]^+$; found, 461.267 $[M + H]^+$.

*6-(3-(3-(Dimethylamino)-1-phenylpropoxy)phenyl)-2-(ethylamino)-9-methyl-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]diazepin-5-one (**32**).* To a solution of **31a** (57 mg, 0.124 mmol) in DCE (3 mL), paraformaldehyde (16 mg, 0.495 mmol), DIPEA (0.108 mL, 0.62 mmol), $NaBH(OAc)_3$ (105 mg, 0.49 mmol), and AcOH (7 μL, 0.12 mmol) were added, and the reaction mixture was stirred at rt for 70 h. $NaHCO_3$ aqueous saturated solution was added, extracted with DCM, washed with brine, dried over Na_2SO_4, filtered, and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 20% MeOH, afforded **32** (63 mg, quant yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.50 (bs, 1H), 7.40−7.38 (m, 2H), 7.34−7.30 (m, 2H), 7.26−7.18 (m, 2H), 6.84−6.83 (m, 1H), 6.81−6.76 (m, 2H), 5.31 (dd, J = 4.7, 7.9 Hz, 1H), 3.85−3.84 (m, 2H), 3.69−3.67 (m, 2H), 3.42 (q, J = 7.1, 16.0 Hz, 2H), 3.21 (s, 3H), 2.59−2.41 (m, 2H), 2.24 (s, 6H), 2.21−2.13 (m, 1H), 2.05−1.96 (m, 1H), 1.21 (t, J = 7.1 Hz, 3H). HPLC, method 1: RT, 4.97 min; purity, 96.1%. HRMS calcd for $C_{27}H_{35}N_6O_2$, 475.2816 $[M + H]^+$; found, 475.2817 $[M + H]^+$.

*2-(Ethylamino)-N-methyl-N-(3-(3-(methylamino)-1-phenylpropoxy)phenyl)pyrimidine-5-carboxamide (**34**).* To a solution of **37** (50 mg, 0.27 mmol) and **27-1-meta** (213 mg, 0.41 mmol) in dioxane (3 mL), CuI (16 mg, 0.08 mmol), K_3PO_4 (118 mg, 0.55 mmol), and N^1,N^2-dimethylethane-1,2-diamine (9 μL, 0.08 mmol) were added, and the mixture was heated at 110 °C under an Ar atmosphere for 16 h. The reaction mixture was cooled to rt, and the solvent was removed under vacuum to give a crude product that was purified by flash chromatography, silica gel, gradient DCM to 25% MeOH, to afford *tert*-butyl (3-(3-(2-(ethylamino)-*N*-methylpyrimidine-5-carboxamido)phenoxy)-3-phenylpropyl)(methyl)carbamate (51 mg, 35% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.65 (s, 2H), 7.34−7.19 (m, 5H), 6.81−6.74 (m, 2 H), 6.72 (m, 1H), 6.13 (m, 1H), 5.10 (dd, J = 4.2, 8.1 Hz, 1H), 3.97−3.83 (m, 2H), 3.47−3.35 (m, 2H), 2.97 (d, J = 4.8 Hz, 3H), 2.84 (s, 3H), 2.23−2.02 (m, 2H), 1.40 (s, 9H), 1.12 (t, J = 7.2 Hz, 3H).

To a solution of the previous compound (40 mg, 0.07 mmol) in dioxane (0.2 mL), HCl (4 M in dioxane, 0.27 mL, 1.07 mmol) was added, and the mixture was stirred at rt for 30 min. The reaction mixture was concentrated to dryness under vacuum. To obtain the free base, DCM was added and washed with Na_2CO_3 (10% aqueous solution), and the aqueous phase was extracted with DCM. The combined organic layers were concentrated under vacuum to afford **34** (30 mg, quant yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.65 (s, 2H), 7.38−7.36 (m, 2H), 7.33−7.30 (m, 2H), 7.25−7.22 (m, 2H), 6.86−6.84 (m, 1H), 6.75−6.74 (m, 2H), 5.31 (dd, J = 4.7, 8.0 Hz, 1H), 3.99−3.88 (m, 2H), 2.88 (s, 3H), 2.75−2.66 (m, 2H), 2.36 (s, 3H), 2.22−2.15 (m, 1H), 2.06−1.99 (m, 1H), 1.08 (t, J = 7.2 Hz, 3H). HPLC, method 1: RT, 5.48 min; purity, 97.5%. HRMS calcd for $C_{24}H_{30}N_5O_2$, 420.2394 $[M + H]^+$; found, 420.2380 $[M + H]^+$.

*2-(Ethylamino)-N-methylpyrimidine-5-carboxamide (**37**).* To a solution of ethyl 2-(methylthio)pyrimidine-5-carboxylate (**36**, 110 mg, 0.55 mmol) in DCM (4 mL), *m*-CPBA (166 mg, 0.72 mmol) was added, and the mixture was stirred at rt for 1 h. A $NaHCO_3$ aqueous saturated solution was added, and the mixture was extracted with DCM. The organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was removed under vacuum. The residue was dissolved in THF (4 mL), ethylamine (2 M solution in THF, 1.38 mL, 2.77 mmol) was added, and the mixture was stirred at rt for 3 h. The solvent was removed under vacuum. The residue was treated with methylamine (3 mL of a 33% solution in EtOH, 24.19 mmol) and NaCN (3 mg, 0.061 mmol), and the mixture was heated at 100 °C for 16 h. The solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 20% MeOH, afforded **37** (90 mg, 90% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 8.68 (s, 2H), 3.44 (q, J = 7.2 Hz, 2H), 2.89 (s, 3H), 1.22 (t, J = 7.2 Hz, 3H).

*(S)-3-(3-Bromophenoxy)-N-methyl-3-(thiophen-2-yl)propan-1-amine (**40S**).* To a solution of (*S*)-3-(methylamino)-1-(thiophen-2-yl)propan-1-ol (**38S**, 1.2 g, 7.01 mmol) in dimethylacetamide (20 mL), NaH (420 mg, 60% in mineral oil, 10.51 mmol) was added, and the mixture was stirred at rt for 30 min. 1-Bromo-3-fluorobenzene (**39**, 2.45 g, 14.01 mmol) was added, and the mixture was heated at 90 °C for 3 h. Water was added, and the mixture was extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and concentrated under vacuum to afford compound **40S** that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (dd, J = 1.2, 5.1 Hz, 1H), 7.13 (m, 1H), 7.10−7.06 (m, 2H), 7.03 (m, 1H), 6.96 (dd, J = 3.4, 5.0 Hz, 1H), 6.87 (dt, J = 2.2, 7.2 Hz, 1H), 5.57 (dd, J = 5.2, 7.5 Hz, 1H), 2.78 (m, 2H), 2.47 (s, 3H), 2.32 (m, 1H), 2.24 (bs, 1H), 2.13 (m, 1H).

*tert-Butyl (S)-(3-(3-Bromophenoxy)-3-(thiophen-2-yl)propyl)-(methyl)carbamate (**41-1S**).* To a solution of **40S** (1.42 g, 4.38 mmol) in DCM (25 mL) at 0 °C, di-*tert*-butyldicarbonate (1.25 g, 5.73 mmol) was added, and the mixture was stirred at rt for 3 h. Water was added, and the mixture was extracted with DCM, washed with $NaHCO_3$ aqueous saturated solution and brine, and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, afforded **41-1S** (1.3 g, 70% yield global two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 (d, J = 5 Hz, 1H), 7.10 (m, 1H), 7.06 (m, 2H), 7.02 (m, 1H), 6,96 (m, 1H), 6.85 (td, J = 7, 2.5 Hz, 1H), 5.41 (m, 1H), 3.43 (m, 2H), 2.87

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(s, 3H), 2.31 (m, 1H), 2.18 (m, 1H), 1.42 (s, 9H). Chiral SFC analysis, method 2: RT, 1.94 min; 98% ee.

*2-(Trimethylsilyl)ethyl (S)-(3-(3-Bromophenoxy)-3-(thiophen-2-yl)propyl)(methyl)carbamate (**41-2S**).* To a solution of **40S** (2 g, 6.13 mmol) in DCM (14 mL), DIPEA (1.07 mL, 6.13 mmol) and a solution of 4-nitrophenyl (2-(trimethylsilyl)ethyl) carbonate (1.73 g, 6.13 mmol) in DCM (6 mL) were added, and the mixture was stirred at rt for 20 h. The reaction mixture was washed with $NaHCO_3$ aqueous saturated solution and 2 M NaOH until the bright yellow color almost disappeared. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, afforded **41-2S** (2.05 g, 62% yield global two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (m, 1H), 7.11−7.05 (m, 3H), 7.03−6.98 (m, 1H), 6.92 (m, 1H), 6.83 (m, 1H), 5.42 (m, 1H), 4.19−3.93 (m, 2H), 3.57 (m, 1H), 3.37 (m, 1H), 2.89 (m, 3H), 2.29 (m, 1H), 2.16 (m, 1H), 0.99 (m, 1H), 0.81 (m, 1H), 0.03 (s, 9H).

*tert-Butyl (S)-Methyl(3-(3-(9-methyl-2-(methylthio)-5-oxo-5,7,8,9-tetrahydro-6H-pyrimido[4,5-e][1,4]diazepin-6-yl)phenoxy)-3-(thiophen-2-yl)propyl)carbamate (**42-1S**).* To a solution of **13-1** (150 mg, 0.67 mmol) and **41-1S** (371 mg, 0.87 mmol) in dioxane (10 mL), CuI (38 mg, 0.20 mmol), K_3PO_4 (284 mg, 1.34 mmol), and N^1,N^2-dimethylethane-1,2-diamine (22 μL, 0.20 mmol) were added, and the mixture was heated at 100 °C in a sealed tube for 20 h. The reaction mixture was cooled to rt and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, afforded **42-1S** (320 mg, 84% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.78 (s,1H), 7.25 (m, 2H), 7.03 (m, 1H), 6.95 (m, 1H), 6.87 (m, 2H), 6.82 (m, 1H), 5.43 (m, 1H), 3.93 (m, 2H), 3.75 (m, 2H), 3.39 (m, 2H), 3.29 (s, 3H), 2.86 (s, 3H), 2.56 (s, 3H), 2.25 (m, 2H), 1.42 (s, 9H).

*2-(Trimethylsilyl)ethyl (S)-Methyl(3-(3-(9-methyl-2-(methylthio)-5-oxo-5,7,8,9-tetrahydro-6H-pyrimido[4,5-e][1,4]diazepin-6-yl)-phenoxy)-3-(thiophen-2-yl)propyl)carbamate (**42-2S**).* To a solution of **13-1** (350 mg, 1.56 mmol) and **41-2S** (1.1 g, 2.34 mmol) in dioxane (15 mL), CuI (89 mg, 0.47 mmol), K_3PO_4 (662 mg, 3.12 mmol), and N^1,N^2-dimethylethane-1,2-diamine (50 μL, 0.47 mmol) were added, and the mixture was heated at 100 °C in a sealed tube for 20 h. The reaction mixture was cooled to rt and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, afforded **42-2S** (815 mg, 85% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.78 (s, 1H), 7.26 (m, 2H), 7.04 (m, 1H), 6.96 (m, 1H), 6.88 (m, 2H), 6.84 (m, 1H), 5.45 (m, 1H), 4.15 (m, 2H), 3.95 (m, 2H), 3.76 (m, 2H), 3.45 (m, 2H), 3.30 (s, 3H), 2.91 (s, 3H), 2.57 (s, 3H), 2−33 (m, 1H), 2−19 (m, 1H), 1.00 (m, 1H), 0.93 (m, 1H), 0.05 (s, 9H).

*(S)-2-(Ethylamino)-9-methyl-6-(3-(3-(methylamino)-1-(thiophen-2-yl)propoxy)phenyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]diazepin-5-one (**45aS**).* To a solution of **42-1S** (970 mg, 1.70 mmol) in DCM (20 mL), *m*-CPBA (588 mg, 2.55 mmol) was added, and the mixture was stirred at rt for 1 h. $NaHCO_3$ aqueous saturated solution was added, and the mixture was extracted with DCM. The organic layer was washed with brine and dried with Na_2SO_4, and the solvent was removed under vacuum. The crude product was dissolved in THF (15 mL), and ethylamine (2 M solution in THF, 4.26 mL, 8.51 mmol) was added. The mixture was stirred at rt for 20 h. The solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, afforded *tert*-butyl (*S*)-(3-(3-(2-(ethylamino)-9-methyl-5-oxo-5,7,8,9-tetrahydro-6*H*-pyrimido[4,5-*e*][1,4]diazepin-6-yl)phenoxy)-3-(thiophen-2-yl)propyl)(methyl)carbamate (817 mg, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H), 7.23−7.19 (m, 2H), 7.01 (d, J = 3.0 Hz, 1H), 6.93 (dd, J = 3.6, 5.0 Hz, 1H), 6.87−6.85 (m, 2H), 6.78 (ddd, J = 0.8, 2.2, 8.2 Hz, 1H), 5.41 (dd, J = 5.2, 7.8, 1H), 5.16 (bs, 1H), 3.90−3.88 (m, 2H), 3.67−3.65 (m, 2H), 3.50−3.43 (m, 2H), 3.41−3.33 (m, 2H), 3.20 (s, 3H), 2.84 (s, 3H), 2.32−2.26 (m, 1H), 2.20−2.11 (m, 1H), 1.41 (s, 9H), 1.23 (t, J = 7.3 Hz, 3H).

A solution of the previous compound (800 mg, 1.41 mmol) in DCM (15 mL) was added to $ZnBr_2$ (1.9 g, 8.47 mmol, dried under vacuum at 250 °C for 2 h) under an atmosphere of Ar, and the reaction mixture was stirred at rt for 48 h. Water was added and stirred until complete solution with the help of ultrasound irradiation. The layers were separated, and the aqueous phase was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 40% MeOH, afforded a salt that was treated with $NaHCO_3$ aqueous saturated solution and extracted with DCM. The solvent was removed under vacuum to afford **45aS** (345 mg, 52% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.52 (s, 1H), 7.32 (dd, J = 1.1, 5.2 Hz, 1H), 7.24 (t, J = 8.2 Hz, 1H), 7.10 (dd, J = 0.7, 3.5 Hz, 1H), 6.95 (dd, J = 3.5, 5.1 Hz, 1H), 6.92−6.88 (m, 2H), 6.83 (ddd, J = 0.7, 1.7, 8.2 Hz, 1H), 5.67 (dd, J = 5.7, 7.5 Hz, 1H), 3.89−3.87 (m, 2H), 3.73−3.71 (m, 2H), 3.45−3.40 (m, 2H), 3.22 (s, 3H), 2.72−2.66 (m, 2H), 2.36 (s, 3H), 2.36−2.25 (m, 1H), 2.14−2.06 (m, 1H), 1.22 (t, J = 7.2 Hz, 3H). HPLC, method 1: RT, 4.82 min; purity, 99.3%. HRMS calcd for $C_{24}H_{30}N_6O_2S$, 467.2224 $[M + H]^+$; found, 467.2233 $[M + H]^+$.

*2-(Dimethylamino)-9-methyl-6-(3-(3-(methylamino)-1-(thiophen-2-yl)propoxy)phenyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]diazepin-5-one (**45b**).* To a solution of **42-2** (105 mg, 0.17 mmol) in DCM (5 mL), *m*-CPBA (59 mg, 0.26 mmol) was added, and the mixture was stirred at rt for 1 h. $NaHCO_3$ aqueous saturated solution was added, and the mixture was extracted with DCM. The organic layer was washed with brine and dried with Na_2SO_4, and the solvent was removed under vacuum. The crude product was dissolved in THF (1 mL), and dimethylamine (40% solution in water, 0.43 mL) was added. The mixture was stirred at rt for 20 h. The solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 25% MeOH, afforded 2-(trimethylsilyl)ethyl (3-(3-(2-(dimethylamino)-9-methyl-5-oxo-5,7,8,9-tetrahydro-6*H*-pyrimido[4,5-*e*][1,4]diazepin-6-yl)phenoxy)-3-(thiophen-2-yl)-propyl)(methyl)carbamate (90 mg, 86% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.75 (s, 1H), 7.21−7.17 (m, 2H), 7.03−6.68 (m, 1H), 6.93−6.91 (m, 1H), 6.86−6.84 (m, 2H), 6.78−6.74 (m, 1H), 5.44−5.39 (m, 1H), 4.17−4.03 (m, 2H), 3.88−3.86 (m, 2H), 3.65−3.63 (m, 2H), 3.50−3.34 (m, 2H), 3.19 (s, 6H), 3.18 (s, 3H), 2.87 (s, 3H), 2.34−2.24 (m, 1H), 2.21−2.11 (m, 1H), 1.00−0.87 (m, 2H), 0.01 (s, 9H).

To a solution of the previous compound (90 mg, 0.14 mmol) in DMF (4 mL), CsF (112 mg, 0.73 mmol) was added, and the mixture was heated at 90 °C for 90 min. The solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 40% MeOH, afforded **45b** (55 mg, 85% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.58 (s, 1H), 7.32 (dd, J = 1.2 5.1 Hz, 1H), 7.24 (t, 1H), 7.10 (dd, J = 1.1, 3.3 Hz, 1H), 6.95 (dd, J = 3.6, 5.1 Hz, 1H), 6.92−6.88 (m, 2H), 6.83 (ddd, J = 0.7, 1.9, 8.0 Hz, 1H), 5.68 (dd, J = 5.4, 7.5, 1H), 3.88−3.86 (m, 2H), 3.72−3.70 (m, 2H), 3.21 (s, 3H), 3.18 (s, 6H), 2.85−2.72 (m, 2H), 2.41 (s, 3H), 2.35−2.27 (m, 1H), 2.17−2.10 (m, 1H). HPLC, method 1: RT, 4.73 min; purity, 100%. HRMS calcd for $C_{24}H_{31}N_6O_2S$, 467.2224 $[M + H]^+$; found, 467.2230 $[M + H]^+$.

*(S)-2-Amino-9-methyl-6-(3-(3-(methylamino)-1-(thiophen-2-yl)-propoxy)phenyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]-diazepin-5-one (**45cS**).* A similar procedure to that used for the preparation of **45b** was used for the preparation of **45cS**, using, in step 1, ammonia 30% solution in water under MW irradiation at 100 °C for 30 min. ^{1}H NMR (400 MHz, CD_3OD): δ 8.52 (s, 1H), 7.30 (d, J = 4.9 Hz, 1H), 7.23 (t, 1H), 7.09 (d, J = 3.6 Hz, 1H), 6.94−6.87 (m, 3H), 6.81 (d, J = 7.9 Hz, 1H), 5.66 (dd, J = 5.8, 7.7, 1H), 3.85−3.83 (m, 2H), 3.68−3.66 (m, 2H), 3.17 (s, 3H), 2.75−2.62 (m, 2H), 2.35 (s, 3H), 2.33−2.24 (m, 1H), 2.14−2.06 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 168.08, 163.19, 162.62, 158.73, 158.41, 144.71, 144.59, 129.64, 126.31, 125.38, 124.98, 118.88, 114.54, 114.50, 103.44, 74.57, 54.53, 49.64, 47.55, 37.84, 37.62, 34.78. HRMS calcd for $C_{22}H_{26}N_6O_2S$, 439.1900 $[M + H]^+$; found, 439.1902 $[M + H]^+$.

*(S)-9-Methyl-6-(3-(3-(methylamino)-1-(thiophen-2-yl)propoxy)-phenyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]diazepin-5-one (**46S**).* A mixture of **42-2S** (1 g, 1.63 mmol) and 5% Pd/C (139 mg, 0.065 mmol) in THF (15 mL) was cooled at 0 °C, and triethylsilane (1.3 mL, 8.15 mmol) was added. The reaction mixture was stirred at 0

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°C in a sealed tube for 5 min and then at rt for 4 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under vacuum. Purification by flash chromatography, silica gel, gradient hexane to 100% acetone, afforded 2-(trimethylsilyl)ethyl (*S*)-methyl(3-(3-(9-methyl-5-oxo-5,7,8,9-tetrahydro-6*H*-pyrimido[4,5-*e*][1,4]diazepin-6-yl)phenoxy)-3-(thiophen-2-yl)propyl)carbamate (750 mg, 81% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (s, 1H), 8.69 (s, 1H), 7.27−7.23 (m, 2H), 7.04 (m, 1H), 6.95 (m, 1H), 6.88 (m, 2H), 6.84 (m, 1H), 5.45 (m, 1H), 4.20−4.05 (m, 2H), 3.94 (m, 2H), 3.77 (m, 2H), 3.51−3.39 (m, 2H), 3.28 (s, 3H), 2.90 (s, 3H), 2.32 (m, 1H), 2.19 (m, 1H), 0.99 (m, 1H), 0.92 (m, 1H), 0.04 (s, 9H).

To a solution of the previous compound (720 mg, 1.27 mmol) in DMF (12 mL), CsF (963 mg, 6.34 mmol) was added, and the mixture was heated at 90 °C for 90 min. Water was added and stirred until complete solution with the help of ultrasound irradiation. DCM was added, and the mixture was filtered through a pad of Celite and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 35% MeOH/NH_3 aq (1:0.01), afforded **46S** (510 mg, 87% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.72 (s, 1H), 8.56 (s, 1H), 7.32 (dd, J = 0.9, 4.9 Hz, 1H), 7.27 (t, 1H), 7.10 (d, J = 3.3 Hz, 1H), 6.96−6.91 (m, 3H), 6.87 (d, J = 8.0 Hz, 1H), 5.68 (dd, J = 5.7, 7.7, 1H), 3.95−3.93 (m, 2H), 3.84−3.82 (m, 2H), 3.28 (s, 3H), 2.76−2.63 (m, 2H), 2.36 (s, 3H), 2.34−2.24 (m, 1H), 2.15−2.06 (m, 1H). HPLC, method 1: RT, 4.38 min; purity, 99.2%. HRMS calcd for $C_{22}H_{25}N_5O_2S$, 424.1802 $[M + H]^+$; found, 424.1808 $[M + H]^+$.

*(S)-2,9-Dimethyl-6-(3-(3-(methylamino)-1-(thiophen-2-yl)-propoxy)phenyl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-e][1,4]-diazepin-5-one (**47S**).* To a solution of **42-1S** (250 mg, 0.44 mmol) in DCM (7 mL), *m*-CPBA (151 mg, 0.66 mmol) was added, and the mixture was stirred at rt for 1 h. $NaHCO_3$ aqueous saturated solution was added, and the mixture was extracted with DCM. The organic layer was washed with brine and dried with Na_2SO_4, and the solvent was removed under vacuum. The crude product was dissolved in THF (7 mL) and cooled at 0 °C, and MeMgBr (3 M solution in Et_2O, 0.176 mL, 0.52 mmol) was added. The mixture was stirred at 0 °C for 1 h. Some drops of water were added, and the solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient cyclohexane to 80% acetone, afforded *tert*-butyl (*S*)-(3-(3-(2,9-dimethyl-5-oxo-5,7,8,9-tetrahydro-6*H*-pyrimido[4,5-*e*][1,4]-diazepin-6-yl)phenoxy)-3-(thiophen-2-yl)propyl)(methyl)carbamate (160 mg, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.87 (s,1H), 7.23 (m, 2H), 7.03 (m, 1H), 6.94 (m, 1H), 6.87 (m, 2H), 6.82 (m, 1H), 5.43 (m, 1H), 3.91 (m, 2H), 3.73 (m, 2H), 3.38 (m, 2H), 3.27 (s, 3H), 2.85 (s, 3H), 2.57 (s, 3H), 2.30 (m, 1H), 2.18 (m, 1H), 1.42 (s, 9H).

A solution of the previous compound (160 mg, 0.30 mmol) in DCM (5 mL) was added to $ZnBr_2$ (402 mg, 1.78 mmol, dried under vacuum at 250 °C for 2 h) under an atmosphere of Ar, and the reaction mixture was stirred at rt for 24 h. Water was added, and the mixture was stirred until the solids completely dissolved with the help of ultrasound irradiation. The layers were separated, and the aqueous phase was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 35% MeOH, afforded a salt that was treated with $NaHCO_3$ aqueous saturated solution and extracted with DCM. The solvent was removed under vacuum to afford **47S** (44 mg, 31% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.74 (s, 1H), 7.40 (dd, J = 1.2, 5.0 Hz, 1H), 7.34 (t, 1H), 7.18 (dd, J = 1.1, 3.5 Hz, 1H), 7.04−6.98 (m, 3H), 6.94 (ddd, J = 0.8, 1.9, 7.9 Hz, 1H), 5.76 (dd, J = 5.7, 7.5 Hz, 1H), 4.01−3.99 (m, 2H), 3.90−3.88 (m, 2H), 3.36 (s, 3H), 2.84−2.71 (m, 2H), 2.58 (s, 3H), 2.44 (s, 3H), 2.42−2.31 (m, 1H), 2.21−2.14 (m, 1H). HPLC, method 1: RT, 4.50 min; purity, 96.1%. HRMS calcd for $C_{23}H_{27}N_5O_2S$, 438.1957 $[M + H]^+$; found, 438.1959 $[M + H]^+$.

*tert-Butyl (2-(2,6-Dichloronicotinamido)ethyl)(methyl)-carbamate (**52-2**).* To a solution of 2,6-dichloronicotinoyl chloride (**50-2**, 2.45 g, 11.67 mmol) in THF (18 mL) at 0 °C, a solution of *tert*-butyl (2-aminoethyl)(methyl)carbamate (**51**, 1.90 g, 10.90 mmol) in THF (27 mL) and Et_3N (5.64 mL, 40.50 mmol) were added. The mixture was stirred at 0 °C for 10 min and then at rt for 2.5 h. Water was added, the mixture was extracted with DCM, the DCM extract was isolated and dried over Na_2SO_4, and the solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient cyclohexane to 100% ethyl acetate, afforded **52-2** (2.9 g, 76% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 8.01 (d, J = 7.83 Hz, 1H), 7.34 (d, J = 7.83 Hz, 1H), 3.60−3.64 (m, 2H), 3.50−3.54 (m, 2H), 2.93 (s, 3H), 1.43 (s, 9H).

*2,6-Dichloro-N-(2-(methylamino)ethyl)nicotinamide Hydrochloride (**53-2**).* To a solution of **52-2** (2.9 g, 8.33 mmol) in dioxane (15 mL), HCl (4 M solution in dioxane, 31.2 mL, 125 mmol) was added, and the mixture was stirred at rt for 2 h. The reaction mixture was concentrated to dryness under vacuum to afford **53-2** (2.3 g, quant yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (d, J = 8.01 Hz, 1H), 7.56 (d, J = 8.01 Hz, 1H), 3.72 (t, J = 6.02 Hz, 2H), 3.28 (t, J = 6.02 Hz, 2H), 2.79 (s, 3H).

*8-Chloro-1-methyl-1,2,3,4-tetrahydro-5H-pyrido[2,3-e][1,4]-diazepin-5-one (**54-2**).* To a mixture of **53-2** (1.2 g, 3.74 mmol) and CsF (2.84 g, 18.69 mmol) in DMF (125 mL) under an Ar atmosphere, Et_3N (1.25 mL, 8.97 mmol) was added, and the mixture was heated at 75 °C for 16 h. The reaction mixture was cooled to rt, and the solvent was removed under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 40% MeOH, afforded **54-2** (729 mg, 92% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (d, J = 8.0 Hz, 1H), 7.26 (bs, 1H), 6.69 (d, J = 8.0 Hz, 1H), 3.65 (m, 2 H), 3.54 (m, 2H), 3.19 (s, 3H).

*8-(Ethylamino)-1-methyl-1,2,3,4-tetrahydro-5H-pyrido[2,3-e]-[1,4]diazepin-5-one (**55-2**).* A mixture of **54-2** (95 mg, 0.45 mmol) and ethylamine (70% solution in water, 2.75 mL, 34.1 mmol) was irradiated with microwaves at 130 °C for 2 h. The solvent was removed under vacuum, and the residue was purified by flash chromatography, silica gel, gradient DCM to 40% MeOH, to afford **55-2** (84 mg, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, J = 8.5 Hz, 1H), 6.19 (bs, 1H), 5.85 (d, J = 8.5 Hz, 1H), 4.58 (bs, 1H), 3.58 (m, 2 H), 3.49 (m, 2H), 3.38 (m, 2H), 3.17 (s, 3H), 1.26 (t, J = 7.2 Hz, 3H).

A similar procedure was used for the preparation of **55-1** starting from **50-1**.

*(S)-8-(Ethylamino)-1-methyl-4-(3-(3-(methylamino)-1-(thiophen-2-yl)propoxy)phenyl)-1,2,3,4-tetrahydro-5H-pyrido[2,3-e]-[1,4]diazepin-5-one (**57S**).* A mixture of CuI (18 mg, 0.09 mmol) and N^1,N^2-dimethylethane-1,2-diamine (10 μL, 0.09 mmol) in dioxane (0.25 mL) was stirred at rt for 20 min. To this mixture, a solution of **55-2** (41 mg, 0.19 mmol) and **41-2S** (96 mg, 0.19 mmol) in a mixture of dioxane (1.25 mL) and DMSO (0.2 mL) and K_3PO_4 (119 mg, 0.56 mmol) were added. The reaction mixture was heated at 130 °C in a sealed tube for 20 h. The reaction mixture was cooled at rt and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient hexane to 100% ethyl acetate, afforded 2-(trimethylsilyl)ethyl (*S*)-(3-(3-(8-(ethylamino)-1-methyl-5-oxo-1,2,3,5-tetrahydro-4*H*-pyrido[2,3-*e*][1,4]diazepin-4-yl)phenoxy)-3-(thiophen-2-yl)propyl)(methyl)carbamate (51 mg, 45% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, J = 8.5 Hz, 1H), 7.27−7.19 (m, 2H), 7.03 (br, 1H), 6.95−6.90 (m, 3H), 6.78 (d, J = 8.0 Hz, 1H), 5.87 (d, J = 8.4 Hz, 1H), 5.46−5.42 (m, 1H), 4.63−4.59 (m, 1H), 4.17−4.10 (m, 2H), 3.91−3.88 (m, 2H), 3.60−3.57 (m, 2H), 3.45−3.31 (m, 4H), 3.09 (s, 3H), 2.90 (s, 3H), 2.35−2.28 (m, 1H), 2.22−2.12 (m, 1H), 1.26 (t, J = 7.2 Hz, 3H), 0.97 (bs, 2H), 0.04 (s, 9H).

To a solution of the previous compound (51 mg, 0.08 mmol) in DMF (0.6 mL), CsF (64 mg, 0.42 mmol) was added, and the mixture was irradiated with MW at 90 °C for 90 min. The reaction mixture was cooled at rt, filtered through a pad of Celite, and concentrated under vacuum to afford **57S** (38 mg, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, J = 8.5 Hz, 1H), 7.22−7.18 (m, 2H), 7.01 (d, J = 3.4 Hz, 1H), 6.94−6.87 (m, 3H), 6.79 (dd, J = 2.3, 8.4 Hz, 1H), 5.86 (d, J = 8.5 Hz, 1H), 5.55 (dd, J = 5.6, 7.6 Hz, 1H), 4.59 (br, 1H), 3.89−3.87 (m, 2H), 3.58−3.56 (m, 2H), 3.39−3.32 (m, 2H), 3.07 (s, 3H), 2.77−2.67 (m, 2H), 2.41 (s, 3H), 2.32−2.21 (m, 1H), 2.12−2.04 (m, 1H), 1.24 (t, 3H). HPLC, method 1: RT, 4.84 min; purity,

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96.0%. HRMS calcd for $C_{25}H_{31}N_5O_2S$, 466.2271 $[M + H]^+$; found, 466.2276 $[M + H]^+$.

A similar procedure was used for the preparation of **56S**, **59S**, **61S**, **63S**, **65**, **67**, **69**, **71R**, and **71S** from the corresponding starting materials. In some examples, purification by flash chromatography was needed (silica gel, gradient DCM to 40% MeOH).

*(S)-1-Methyl-4-(3-(3-(methylamino)-1-(thiophen-2-yl)propoxy)-phenyl)-3,4-dihydro-1H-pyrido[2,3-e][1,4]diazepin-5(2H)-one (**59S**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (dd, J = 2.1, 4.8 Hz, 1H), 8.04 (dd, J = 2.1, 7.6 Hz, 1H), 7.27−7.22 (m, 2H), 7.02 (d, J = 3.1 Hz, 1 H), 6.96−6.91 (m, 3H), 6.85 (dd, J = 2.3, 8.2 Hz, 1H), 6.80 (dd, J = 4.8, 7.8 Hz, 1H), 5.57 (dd, J = 5.4, 7.7 Hz, 1H), 3.90−3.87 (m, 2H), 3.61−3.58 (m, 2H), 3.08 (s, 3H), 2.77−2.67 (m, 2H), 2.42 (s, 3H), 2.35−2.20 (m, 1H), 2.14−2.03 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.78, 158.63, 155.56, 151.22, 144.97, 143.72, 140.87, 129.85, 126.59, 124.89, 124.83, 119.64, 118.90, 114.68, 114.51, 114.34, 74.99, 56.81, 49.17, 48.17, 38.76, 38.75, 36.53. HRMS calcd for $C_{23}H_{26}N_4O_2S$, 423.1853 $[M + H]^+$; found, 423.1849 $[M + H]^+$.

*1-Methyl-1,2,3,4-tetrahydro-5H-pyrido[2,3-e][1,4]diazepin-5-one (**58**).* To a solution of **54-2** (550 mg, 2.60 mmol) in THF (12 mL), Pd/C (5% wt, 221 mg, 0.10 mmol) was added, and the mixture was cooled at 0 °C in a sealed tube. Triethylsilane (1.51 g, 13.0 mmol) was added, and the reaction mixture was stirred at 0 °C for 5 min and then at rt for 16 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 30% MeOH, afforded **58** (378 mg, 82% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (dd, J = 2.0, 4.6 Hz, 1H), 8.21 (dd, J = 2.0, 7.6 Hz, 1H), 7.01 (bs, 1H), 6.75 (dd, J = 4.6, 7.6 Hz, 1H), 3.62 (m, 2 H), 3.54 (m, 2H), 3.16 (s, 3H).

*1,8-Dimethyl-1,2,3,4-tetrahydro-5H-pyrido[2,3-e][1,4]diazepin-5-one (**60**).* To a mixture of **54-2** (50 mg, 0.23 mmol), methylboronic acid (16 mg, 0.27 mmol), $Pd(Ph_3)_4$ (27 mg, 0.024 mmol), and K_2CO_3 (98 mg, 0.71 mmol) under Ar, degassed dioxane (1.2 mL) was added, and the mixture was heated at 130 °C in a sealed tube for 48 h. The reaction mixture was cooled to rt and filtered through a pad of Celite. The filtrate was concentrated under vacuum, and the residue was purified by flash chromatography, silica gel, gradient hexane to 100% acetone, to afford **60** (45 mg, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, J = 7.8 Hz, 1H), 6.61 (d, J = 7.8 Hz, 1H), 6.56 (bs, 1H), 3.61 (m, 2 H), 3.53 (m, 2H), 3.19 (s, 3H), 2.45 (s, 3H).

*8-Methoxy-1-methyl-1,2,3,4-tetrahydro-5H-pyrido[2,3-e][1,4]-diazepin-5-one (**62**).* To a solution of **54-2** (500 mg, 2.36 mmol) in MeOH (17 mL), NaOMe (766 mg, 14.17 mmol) was added, and the mixture was irradiated with MW at 110 °C for 2 h. The solvent was removed under vacuum, and the residue was purified by flash chromatography, silica gel, gradient DCM to 30% MeOH, to afford **62** (440 mg, 90% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.09 (d, J = 8.5 Hz, 1H), 6.10 (d, J = 8.5 Hz, 1H), 3.91 (s, 3H), 3.63 (m, 2 H), 3.47 (m, 2H), 3.22 (s, 3H).

*1-Methyl-1,2,3,4-tetrahydro-5H-pyrido[3,2-e][1,4]diazepin-5-one (**64**).* To a solution of ethyl 3-fluoropicolinate (190 mg, 1.12 mmol) in dimethylacetamide (2.2 mL), K_2CO_3 (310 mg, 2.24 mmol) and N^1-methylethane-1,2-diamine (83 mg, 1.12 mmol) were added, and the mixture was heated at 150 °C in a sealed tube for 64 h. The reaction mixture was cooled at rt and concentrated under vacuum. Purification by flash chromatography, silica gel, gradient DCM to 20% MeOH, afforded compound **64** (56 g, 28% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.33 (m, 1H), 7.98 (bs, 1H), 7.32 (m, 1H), 7.27 (m, 1H), 3.45 (m, 2H), 3.37 (m, 2H), 2.89 (s, 3H).

A similar procedure was used for the preparation of **66** from methyl 3-fluoroisonicotinate.

*1-Methyl-1,2,3,4-tetrahydro-5H-benzo[e][1,4]diazepin-5-one (**68**).* To a suspension of 1,2,3,4-tetrahydro-5*H*-benzo[*e*][1,4]-diazepin-5-one (**72**, 410 mg, 2.53 mmol) in DCE (20 mL), DIPEA (653 mg, 5.06 mmol), paraformaldehyde (296 mg, 9.35 mmol), $NaBH(OAc)_3$ (1.98 g, 9.35 mmol), and acetic acid (152 mg, 2.53 mmol) were added, and the reaction mixture was stirred at rt for 48 h. $NaHCO_3$ aqueous saturated solution was added, and the mixture was extracted with DCM. The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed under vacuum. The residue was treated with diethyl ether, and the solid obtained was filtered and washed with diethyl ether to afford **68** (410 mg, 92% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (bs, 1H), 7.67 (m, 1H), 7.38 (m, 1H), 6.98 (m, 1H), 6.89 (m, 1H), 3.36 (m, 2H), 3.30 (m, 2H), 2.84 (s, 3H).

NET Modeling. The human sequence of the sodium-dependent noradrenaline transporter (spP23975) was aligned to the SERT sequences of the crystallized structures 5I6X, 5I6Z, 5I71, 5I73, 5I74, and 5I75,[43] and 100 homology models were generated using the MODELLER[47] program within Discovery Studio.[48] The model with the lower PDF total energy was selected, and its loops were optimized with MODELLER using the DOPE method. The best homology model obtained was typed with CHARMM, residue p*K*'s were calculated, the protein was ionized at pH 7, and compound **31aS** was docked with LibDock,[49] defining the binding site as an 8 Å sphere centered on the paroxetine structure of the aligned 5I6X crystal structure. The obtained complex was inserted into an SPC−POPC orthorhombic box, using the Maestro's system builder,[50] with a 10 Å buffer between the system and the edge of the box, and sodium and chloride ions were added to neutralize and reach a final concentration of 0.15 M. Both systems were typed with the OPLS force field and equilibrated by a standard eight-step protocol for transmembrane proteins, and finally, a 1 μs production simulation in the NPgT ensemble was run and analyzed with Desmond.[51]

Human $\alpha2\delta$-1 Subunit of Cav2.2 Calcium Channel Assay. Human $\alpha2\delta$-1-enriched membranes (3 μg) from hamster tumor CHO-K1 cells (human Cav2.2/β3/$\alpha2\delta$ calcium channel cell line, catalog #CT6159, ChanTest) were incubated with 15 nM radiolabeled [^{3}H]-gabapentin in assay buffer containing HEPES/KOH 10 mM (pH 7.4). Nonspecific binding (NSB) was measured by adding 10 μM pregabalin. The final volume was 100 μL. The binding of the tested compounds was measured at five different concentrations per duplicate, to determine affinity values (K_i). After 60 min of incubation at 27 °C, the binding reaction was terminated by filtering through MultiScreen GF/C (Millipore) presoaked in 0.5% polyethyleneimine in a vacuum manifold station followed by three washes with ice-cold filtration buffer containing 50 mM Tris−HCl (pH 7.4). Filter plates were dried at 60 °C for 2 h, and 30 μL of scintillation cocktail was added to each well before radioactivity reading. Readings were performed in a TriLux 1450 MicroBeta radioactive counter (PerkinElmer).

Human $\alpha2\delta$-2 Subunit of Cav2.2 Calcium Channel Assay. Human $\alpha2\delta$-2-enriched membranes (6 μg) from human embryonic kidney HEK-293 cells were incubated with 15 nM radiolabeled [^{3}H]-gabapentin in assay buffer containing HEPES/KOH 10 mM (pH 7.4). Nonspecific binding (NSB) was measured by adding 10 μM pregabalin. The final volume was 100 μL. The binding of the tested compounds was measured at five different concentrations, duplicate, to determine affinity values (K_i). After 60 min of incubation at 27 °C, the binding reaction was terminated by filtering through MultiScreen GF/C (Millipore) presoaked in 0.5% polyethyleneimine in a vacuum manifold station followed by three washes with ice-cold filtration buffer containing 50 mM Tris−HCl (pH 7.4). Filter plates were dried at 60 °C for 2 h, and 30 μL of scintillation cocktail was added to each well before radioactivity reading. Readings were performed in a TriLux 1450 MicroBeta radioactive counter (PerkinElmer).

NET Binding Assay. NET-enriched membranes (5 μg) were incubated with 5 nM radiolabeled [^{3}H]-nisoxetine in assay buffer containing 50 mM Tris−HCl, 120 mM NaCl, and 5 mM KCl (pH 7.4). NSB (nonspecific binding) was measured by adding 10 μM desipramine. The binding of the test compound was measured at either one concentration (% inhibition at 1 or 10 μM) or five different concentrations to determine affinity values (K_i). After 60 min of incubation at 4 °C, the binding reaction was terminated by filtering through MultiScreen GF/C (Millipore) presoaked in 0.5% polyethyleneimine in a vacuum manifold station followed by three washes with ice-cold filtration buffer containing 50 mM Tris−HCl and 0.9% NaCl (pH 7.4). Filter plates were dried at 60 °C for 1 h, and 30 μL of

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scintillation cocktail was added to each well before radioactivity reading. Readings were performed in a TriLux 1450 MicroBeta radioactive counter (PerkinElmer).

NET Functional Assay. The day before the experiment, 5000 cells/well were seeded in 384 poly-D-lysine-treated well plates (Corning) in a complete medium and maintained at 37 °C at 5% CO_2. On the experiment day, the cell culture medium was removed and trypan blue (20 μL) was added (600 μM final concentration). This was immediately followed by the application of the test compounds (10 μL/well) at different final concentrations (from 10^{-4} to 10^{-11} M). Then, the plates were left at 37 °C for 20 min. After this time, ASP^+ (4-(4-(dimethylamino) styryl)-*N*-methylpyridinium iodide, 20 μL) was added (10 mM final well concentration). The reaction was incubated for 90 min at 37 °C. Finally, the fluorescence was read with the fluorescent reader EnVision (PerkinElmer).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01867.

Analytical data for all the final compounds and intermediates and HPLC traces for final compounds (PDF)

Molecular formula strings of compounds (CSV)

NET homology data for compound **31aS** (PDB)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADME, absorption, distribution, metabolism, and excretion; ASP^+, 4-(4-(dimethylamino)styryl)-*N*-methylpyridinium iodide; $Ca_v\alpha2\delta$-1, $\alpha2\delta$-1 subunit of voltage-gated calcium channels; CNS, central nervous system; FDA, Food and Drug Administration; hERG, human ether-a-go-go-related gene; MAP, 3-methylamino-1-phenylpropoxy; MAT, 3-methylamino-1-thiophenylpropoxy; NE, norepinephrine; NET, norepinephrine transporter; NRI, norepinephrine reuptake inhibitor; PI, positive ionizable; rhCYP, recombinant human cytochrome P450; SAR, structure−activity relationship; SERT, serotonin transporter; SNRI, serotonin and norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; Teoc, trimethylsilylethoxycarbonyl; VGCC, voltage-gated calcium channels

■ REFERENCES

(1) Turk, D. C.; Wilson, H. D.; Cahana, A. Treatment of chronic non-cancer pain. *Lancet* **2011**, *377*, 2226−2235.

(2) Sinatra, R. Causes and consequences of inadequate management of acute pain. *Pain Med* **2010**, *11*, 1859−1871.

(3) Raffa, R. B.; Pergolizzi, J. V., jr.; Tallarida, R. J. The determination and application of fixed-dose analgesic combinations for treating multimodal pain. *J. Pain* **2010**, *11*, 701−709.

(4) Chou, R.; Gordon, D. B.; de Leon-Casasola, O. A.; Rosenberg, J. M.; Bickler, S.; Carter, T.; Cassidy, L.; Chittenden, E. H.; Degenhardt, E.; Griffith, S.; Manworren, M. R.; McCarberg, B.; Montgomery, R.; Murphy, J.; Perkal, M. F.; Suresh, S.; Sluka, K.; Strassels, S.; Thirlby, R.; Viscusi, E.; Walco, G. A.; Warner, L.; Weisman, S. J.; Wu, C. L.

Management of postoperative pain: a clinical practice guideline from the American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Committee, and Administrative Council. *J. Pain* **2016**, *17*, 131−157.
(5) Mao, J.; Gold, M. S.; Backonja, M. M. Combination drug therapy for chronic pain: a call for more clinical studies. *J. Pain* **2011**, *12*, 157−166.
(6) Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* **2005**, *48*, 6523−6543.
(7) Zamponi, G. W.; Striessnig, J.; Koschak, A.; Dolphin, A. C. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. *Pharmacol. Rev.* **2015**, *67*, 821−870.
(8) Davies, A.; Hendrich, J.; Van Minh, A. T.; Wratten, J.; Douglas, L.; Dolphin, A. C. Functional biology of the alpha(2)delta subunits of voltage-gated calcium channels. *Trends Pharmacol. Sci.* **2007**, *28*, 220−228.
(9) Li, Z.; Taylor, C. P.; Weber, M.; Piechan, J.; Prior, F.; Bian, F.; Cui, C.; Hoffman, D.; Donevan, S. Pregabalin is a potent and selective ligand for $\alpha 2\delta$-1 and $\alpha 2\delta$-2 calcium channel subunits. *Eur. J. Pharmacol.* **2011**, *667*, 80−90.
(10) Perret, D.; Luo, Z. D. Targeting voltage-gated calcium channels for neuropathic pain management. *Neurotherapeutics* **2009**, *6*, 679−692.
(11) Derry, S.; Cording, M.; Wiffen, P. J.; Law, S.; Phillips, T.; Moore, R. A. Pregabalin for pain in fibromyalgia in adults. *Cochrane Database Syst. Rev.* **2016**, *29*, CD011790.
(12) Taylor, C. P.; Angelotti, T.; Fauman, E. Pharmacology and mechanism of action of pregabalin: The calcium channel α_2-δ ($alpha_2$−delta) subunit as a target for antiepileptic Drug Discovery. *Epilepsy Res.* **2007**, *73*, 137−150.
(13) Faulkner, M. A. Use of $\alpha 2\delta$ ligands for restless legs syndrome/Willis Ekbom Disease. *CNS Drugs* **2018**, *32*, 149−159.
(14) Field, M. J.; Li, Z.; Schwarz, J. B. Ca2+ channel alpha2-delta ligands for the treatment of neuropathic pain. *J. Med. Chem.* **2007**, *50*, 2569−2575.
(15) Domon, Y.; Arakawa, N.; Inoue, T.; Matsuda, F.; Takahashi, M.; Yamamura, N.; Kai, K.; Kitano, Y. Binding characteristics and analgesic effects of mirogabalin, a novel ligand for the $\alpha 2\delta$ subunit of voltage-gated calcium channels. *J. Pharmacol. Exp. Ther.* **2018**, *365*, 573−582.
(16) Deeks, E. D. Mirogabalin: First global approval. *Drugs* **2019**, *79*, 463−468.
(17) Lim, J.; Stock, N.; Pracitto, R.; Boueres, J. K.; Munoz, B.; Chaudhary, A.; Santini, A. M.; Orr, K.; Schaffhauser, H.; Bezverkov, R. E.; Aiyar, J.; Venkatraman, S. N-Acridin-9-yl-butane-1,4-diamine derivatives: high-affinity ligands of the alpha2delta subunit of voltage gated calcium channels. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1913−1916.
(18) Lebsack, A. D.; Gunzner, J.; Wang, B.; Pracitto, R.; Schaffhauser, H.; Santini, A.; Aiyar, J.; Bezverkov, R.; Munoz, B.; Liu, W.; Venkatraman, S. Identification and synthesis of [1,2,4]-triazolo[3,4-*a*]phthalazine derivatives as high-affinity ligands to the $\alpha_2\delta$-1 subunit of voltage gated calcium channel. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2463−2467.
(19) Stearns, B. A.; Anker, N.; Arruda, J. M.; Campbell, B. T.; Chen, C.; Cramer, M.; Hu, T.; Jiang, X.; Park, K.; Ren, K. K.; Sablad, M.; Santini, A.; Schaffhauser, H.; Urban, M. O.; Munoz, B. Synthesis and biological evaluation of 6-aryl-6*H*-pyrrolo[3,4-*d*]pyridazine derivatives: high-affinity ligands to the alpha 2 delta subunit of voltage gated calcium channels. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1295−1298.
(20) Hu, T.; Stearns, B. A.; Campbell, B. T.; Arruda, J. M.; Chen, C.; Aiyar, J.; Bezverkov, R. E.; Santini, A.; Schaffhauser, H.; Liu, W.; Venkatraman, S.; Munoz, B. Synthesis and biological evaluation of 6-aryl-6*H*-pyrrolo[3,4-*d*]pyridazine derivatives as high-affinity ligands of the alpha(2)delta subunit of voltage-gated calcium channels. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2031−2034.
(21) Chen, C.; Stearns, B.; Hu, T.; Anker, N.; Santini, A.; Arruda, J. M.; Campbell, B. T.; Datta, P.; Aiyar, J.; Munoz, B. Expedited SAR study of high-affinity ligands to the $\alpha 2\delta$ subunit of voltage-gated calcium channels: generation of a focused library using a solution-phase Sn2Ar coupling methodology. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 746−749.
(22) Myatt, J. W.; Healy, M. P.; Bravi, G. S.; Billinton, A.; Johnson, C. N.; Matthews, K. L.; Jandu, K. S.; Meng, W.; Hersey, A.; Livermore, D. G.; Douault, C. B.; Witherington, J.; Bit, R. A.; Rowedder, J. E.; Brown, J. D.; Clayton, N. M. Pyrazolopyridazine alpha-2-delta-1 ligands for the treatment of neuropathic pain. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4683−4688.
(23) Otsubo, N.; Tsukumo, Y.; Uchida, K.; Matsymmoto, Y.; Iida, K.; Takada, H.; Takizawa, F.; Arai, H.; Okazaki, S.; Imaizumi, T. Pyrimidodiazepinone Derivatives. US20,100,190,775, 2010.
(24) Otsubo, N.; Okazaki, S.; Tsukumo, Y.; Iida, K.; Nakoji, M. Pyrimidodiazepinone Compounds. US20140171422, 2014.
(25) Ossipov, M. H.; Dussor, G. O.; Porreca, F. Central modulation of pain. *J. Clin. Invest.* **2010**, *120*, 3779−3787.
(26) Hiroki, T.; Suto, T.; Saito, S.; Obata, H. Repeated administration of amitriptyline in neuropathic pain: modulation of the noradrenergic descending inhibitory system. *Anesth. Analg.* **2017**, *125*, 1281−1288.
(27) Hartrick, C. T. Noradrenergic reuptake inhibition in the treatment of pain. *Expert Opin. Invest. Drugs* **2012**, *21*, 1827−1834.
(28) Hughes, S. W.; Hickey, L.; Hulse, R. P.; Lumb, B. M.; Pickering, A. E. Endogenous analgesic action of the pontospinal noradrenergic system spatially restricts and temporally delays the progression of neuropathic pain following tibial nerve injury. *Pain* **2013**, *154*, 1680−1690.
(29) Available from: https://www.fda.gov/consumers/consumer-updates/living-fibromyalgia-drugs-approved-manage-pain. (accessed February 13, 2021).
(30) Dvoracsko, S.; Stefanucci, A.; Novellino, E.; Mollica, A. The design of multitarget ligands for chronic and neuropathic pain. *Future Med. Chem.* **2015**, *7*, 2469−2483.
(31) Bhosle, D.; Bharambe, S.; Gairola, N.; Dhaneshwar, S. S. Mutual prodrug concept: fundamentals and applications. *Indian J. Pharm. Sci.* **2006**, *68*, 286−294.
(32) Shonberg, J.; Scammells, P. J.; Capuano, B. Design strategies for bivalent ligands targeting GPCRs. *Chem. Med. Chem.* **2011**, *6*, 963−974.
(33) Cuevas, F.; Almansa, C. Tetrahydropyrimidodiazepine and tetrahydropyridodiazepine Compounds for Treating Pain and Pain Related Conditions. *PCT Int. Appl.* WO2017191304, 2017.
(34) Almansa, C.; Cuevas, F. New Alcoxyamino Derivatives for Treating Pain and Pain Related Conditions. *PCT Int. Appl.* WO2019081691, 2019.
(35) Yenes, S.; Almansa, C. Compounds Having Multimodal Activity Against Pain. WO2019002173, 2019.
(36) Field, M. J.; Li, Z.; Schwarz, J. B. Ca^{2+} channel α_2-δ ligands for the treatment of neuropathic pain. *J. Med. Chem.* **2007**, *50*, 2569−2575.
(37) (a) Obach, R. S.; Baxter, J. G.; Liston, T. E.; Silber, B. M.; Jones, B. C.; MacIntyre, F.; Rance, D. J.; Wastall, P. The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 46−58. (b) Obach, R. S. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Am. Soc. Pharmacol. Exp. Ther.* **1999**, *27*, 1350−1359.
(38) ((a)) Stresser, D. M. High-throughput Screening of Human Cytochrome P450 inhibitors Using Fluorometric Substrates. Methodology for 25 Enzyme/Substrate Pairs. In *Optimization in Drug Discovery. In vitro Methods*; Yan, Z.; Caldwell, G.W.; Eds. Humana Press: Totowa, New Jersey, 2004; pp. 215−230; (b) Yan, Z.; Caldwell, G. W. Evaluation of Cytochrome P450 inhibition in Human Liver Microsomes. *Optim. Drug Discovery* **2004**, 231−244. ((c)) Drug Interaction Studies − Study Design, Data Analysis, Implications

https://dx.doi.org/10.1021/acs.jmedchem.0c01867
J. Med. Chem. 2021, 64, 2167−2185

for Dosing and Labeling. Center for Drug Evaluation and Research (CDER), Food and Drug Administration, September 2006 and February 2012.
(39) Diaz, G. J.; Daniell, K.; Leitza, S. T.; Martin, R. L.; Su, Z.; McDermott, J. S.; Cox, B. F.; Gintant, G. A. The [^{3}H]-dofetilide binding assay is a predictive screening tool for hERG blockade and proarrhythmia: Comparison of intact cell and membrane preparations and effects of altering $[K^+]_o$. *J. Pharmacol. Toxicol. Methods* **2004**, *50*, 187−199.
(40) Bymaster, F. P.; Beedle, E. E.; Findlay, J.; Gallagher, P. T.; Krushinski, J. H.; Mitchell, S.; Robertson, D. W.; Thompson, D. C.; Wallace, L.; Wong, D. T. Duloxetine (Cymbalta), a dual inhibitor of serotonin and norepinephrine reuptake. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4477−4480.
(41) Andersen, J.; Stuhr-Hansen, N.; Zachariassen, L.; Toubro, S.; Hansen, S. M.; Eildal, J. N.; Bond, A. D.; Bøgesø, K. P.; Bang-Andersen, B.; Kristensen, A. S.; Strømgaard, K. Molecular determinants for selective recognition of antidepressants in the human serotonin and norepinephrine transporters. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 12137−12142.
(42) Wang, H.; Goehring, A.; Wang, K. H.; Penmatsa, A.; Ressler, R.; Gouaux, E. Structural basis for action by diverse antidepressants on biogenic amine transporters. *Nature* **2013**, *503*, 141−145.
(43) Coleman, J. A.; Green, E. M.; Gouaux, E. X-ray structures and mechanism of the human serotonin transporter. *Nature* **2016**, *532*, 334−339.
(44) Razzetti, G.; Allegrini, P.; Pastorello, D. Process for the Synthesis of Pregabalin. US8063244B2, 2009.
(45) Baell, J. B.; Holloway, G. A. New substructure filters for removal of Pan Assay Interference Compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem.* **2010**, *53*, 2719−2740.
(46) Castro, J.; Terricabras, E.; Erra, M.; Navarro, E.; Fonquerna, S.; Cardús, A, Lozoya, M. Azabiphenylaminobenzoic Acid Derivatives as DHODH Inhibitors. WO2009021696, 2009.
(47) Eswar, N.; Webb, B.; Marti−Renom, M. A.; Madhusudhan, M. S.; Eramian, D.; Shen, M.-Y.; Pieper, U.; Sali, A. Comparative protein structure modeling using MODELLER. *Curr. Protoc. Protein Sci.* **2007**, *50*, 5.6.1−5.6.37.
(48) Discovery Studio 16; Dassault Systèmes BIOVIA: San Diego, CA, 2016.
(49) Diller, D. J.; Merz, K. M. High throughput docking for library design and library prioritization. *Proteins* **2001**, *43*, 113−124.
(50) Schrödinger Release 2017-4: Maestro, Schrödinger, LLC, New York, NY, 2020.
(51) Bowers, K. J.; Chow, E.; Xu, H.; Dror, R. O.; Eastwood, M. P.; Gregersen, B. A.; Klepeis, J. L.; Kolossvary, I.; Moraes, M. A.; Sacerdoti, F. D.; Salmon, J. K.; Shan, Y.; Shaw, D. E. Scalable algorithms for Molecular Dynamics Simulations on commodity clusters. *Proceedings of the ACM/IEEE Conference on Supercomputing (SC06)*; Tampa, Florida, 2006, November 11−17.

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