Published in final edited form as:

J Med Chem. 2019 November 14; 62(21): 9600–9617. doi:10.1021/acs.jmedchem.9b01030.

Structural Basis for Achieving GSK-3 β Inhibition with High Potency, Selectivity, and Brain Exposure for Positron Emission Tomography Imaging and Drug Discovery

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmed-chem.9b01030. Additional figures illustrating chemistry and radiochemistry methods, HEK-293T P301S hTau cellular assay, in vitro autoradiography, kinase assays, MST assays, culturing and neural differentiation of human iPSC-derived NPCs, WNT signaling reporter assays, NPC proliferation assay with CellTiter-Glo, high-content neuronal assay for p-CRMP2^{T514}, pTau assay, western blot analysis, rodents and nonhuman primate PET imaging studied, neuropharmacokinetic analysis, and NMR spectra (PDF) Molecular formula strings (XLSX)

Accession Codes

Existing PDBs 5KPK and 5K5N were used for in silico docking studies.

The authors declare no competing financial interest.

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Abstract

Using structure-guided design, several cell based assays, and microdosed positron emission tomography (PET) imaging, we identified a series of highly potent, selective, and brain-penetrant oxazole-4-carboxamide-based inhibitors of glycogen synthase kinase-3 (GSK-3). An isotopologue of our first-generation lead, [³H]PF-367, demonstrates selective and specific target engagement in vitro, irrespective of the activation state. We discovered substantial ubiquitous GSK-3-specific radioligand binding in Tg2576 Alzheimer's disease (AD), suggesting application for these compounds in AD diagnosis and identified [¹¹C]OCM-44 as our lead GSK-3 radiotracer, with optimized brain uptake by PET imaging in nonhuman primates. GSK-3 β -isozyme selectivity was assessed to reveal OCM-51, the most potent (IC₅₀ = 0.030 nM) and selective (>10-fold GSK-3 β /GSK-3 α) GSK-3 β inhibitor known to date. Inhibition of CRMP2^{T514} and tau phosphorylation, as well as favorable therapeutic window against WNT/ β -catenin signaling activation, was observed in cells.

Graphical Abstract

INTRODUCTION

Glycogen synthase kinase-3 α and β (GSK-3 α/β) are ubiquitous multifunctional serine/threonine kinases, which regulate diverse physiological functions in peripheral organs and

the central nervous system (CNS), ranging from metabolism and cell cycle regulation to brain development. Appropriately, balanced GSK-3 regulation and signaling is therefore central to human physiology but often compromised in diseases of diverse etiologies such as diabetes, cancers, and neurological disorders.^{1,2} GSK-3 α/β are remarkably networked enzymes, with involvement in numerous pathways and over one hundred distinct putative substrates.³ Also, unusual among kinases are the fact that GSK-3 α/β regulation occurs via inactivation while resting cells show high levels of catalytic activity. The inactivation of GSK-3 has been well-characterized and revealed primarily to proceed through N-terminus serine phosphorylation catalyzed by kinases such as AKT/PKB, p70RSK, and p90RSK (at serine 21 and serine 9 for GSK-3 α and GSK-3 β , respectively).^{4,5} Of note, GSK-3 α and GSK-3 β present a significant kinase domain homology with one another, especially within the ATP-binding pocket (97% identity).^{6,7} Congruently, GSK-3 α/β also exhibits substantial functional overlap, although some elements of nonredundancy such as GSK-3 β -specific perinatal lethality have been characterized from paralogue-specific Gsk3 knockout model studies, which together support the notion that individual isozyme contributions should be defined carefully.⁷⁻⁹

Several immunohistochemical, cellular, transgenic, and preclinical studies have implicated GSK-3 deregulation as a contributive factor in neurological diseases including Alzheimer's disease (AD) and non-AD tauopathies.¹⁰⁻¹² While in most cases, GSK-3 β has been the central focus in these studies, both GSK-3 α/β overexpression and inhibition/knockdown experiments have demonstrated roles in APP processing and correlated with increased or decreased amyloid- β production, respectively.^{13,14} In turn, amyloid- β injections in rat trigger APP-processing changes and pathological tau phosphorylation linked to GSK-3 β overexpression and activation.¹⁵ The hyperphosphorylation of tau by GSK-3 α/β also suggests a central implication in neurofibrillary tangle formation, however whether an increase in GSK-3 α/β levels or activities alone, rather than parallel changes in phosphatase activities, constitute the causative pathogenic factor in tau hyperphosphorylation and neurofibrillary tangle accumulation remains to be clarified.¹⁶⁻¹⁸ Both inhibition of GSK-3 in transgenic mice-overexpressing human tau and overexpressing conditional Gsk3b mice were associated with changes in hyperphosphorylated tau protein (pTau) levels tightly linked to GSK-3 levels and activity in vivo.^{19,20} Current evidence using human tissue suggests, as with most immunohistochemical cellular and transgenic rodent analyses, increases in total and active GSK-3 β levels in AD compared to healthy controls.²¹⁻²³ However, data gathered in human brains have been largely insufficient to substantiate in vitro preclinical findings while in vivo pharmacological intervention studies in transgenic mice as well as clinical trials aimed at validating GSK-3 in humans have been obscured by the systematic use of promiscuous inhibitors.²⁴⁻²⁶ Indeed, much of the inability to define whether GSK-3 is an actionable target in neurodegenerative diseases in vivo ensue from the lack of suitable molecular tools. To address this, highly potent, selective, and brain penetrant inhibitors of GSK-3 are needed. However, apart from rare exceptions where some of these criteria have been met, so far clinical and preclinical GSK-3 inhibitors have been both sub-optimal regarding potency or brain exposure and have displayed, despite sporadic claims of selectivity in restricted kinase subsets, poor overall kinome selectivity when systematically evaluated in large-scale screening.²⁷⁻³¹ Moreover, only one recent report has described

kinome-selective GSK-3 inhibitors also achieving α/β -isozyme selectivity, however, none of these isozyme-selective compounds exhibit single-digit nanomolar potencies or have been characterized in the CNS tissue.³²

Herein, we used structure-guided design and microdosed positron emission tomography (PET) imaging to develop novel small-molecule inhibitors of GSK-3 achieving high potency, selectivity, and brain exposure. We describe the rational development of the novel GSK-3 β -isozyme selective inhibitor, named herein as OCM-51 (**51**), which to the best of our knowledge, constitutes the most potent and selective GSK-3 inhibitor identified thus far. Biochemical, biophysical, cellular, and brain tissue-based assays collectively were used to confirm highly selective target engagement. Further analysis also demonstrates differential binding profiles in AD transgenic mice brains versus wild-type. Together, our results describe finely characterized candidate inhibitors which can lead to the development of GSK-3 specific therapeutics as well as diagnostic GSK-3 PET molecular probes for neuroimaging.

RESULTS

[³H]PF-367 Engages GSK-3 with High Specificity in Cells and Human Postmortem Brain.

Previously, we reported the discovery of PF-04802367 (PF-367; **1**), a potent type-I oxazole-4-carboxamide dual GSK-3 α/β inhibitor, showing promising ADME properties combined with robust CNS/peripheral pTau and muscle pGS inhibition in vivo.²⁸ Here, we first tested whether the lead compound, PF-367, displayed specific GSK-3 α/β binding in the mammalian brain tissue. We developed [³H]**1** (Figure 1A) and observed, in rat, marmoset and human brains (frontal cortex and hippo-campus), high and nearly ubiquitous total binding in the gray matter, consistent with known GSK-3 α/β mRNA and protein levels (Figures 1B–F and S1B,C). Highest radioligand binding was measured in the cerebral cortex and consistently more pronounced within gray matter regions compared to the white matter in all species (50–60% difference). To confirm that our GSK-3 α/β marker provided saturable signal, homologous competition was performed on adjacent sections using unlabeled **1** at 10 μ M and resulted in significantly reduced binding compared to baseline experiments and overall low levels of nonsaturable binding (>87, 66, and 85% reductions in rat, marmoset, and human cerebral cortices, respectively, Figure 1B,D,E). We then used two structurally unrelated and well-characterized heterologous pan-GSK-3 competitive inhibitors to determine on-target binding selectivity. Single concentration cotreatments with the advanced preclinical inhibitor CHIR99021 and the clinical lead LY2090314 both resulted in substantial decreases in tracer binding similar to unlabeled **1** incubations (Figures 1B–F and S1A). In all species and brain regions, there were no significant discrepancies in blocking effects between **1** and the heterologous probes, indicating that GSK-3 α/β specificity could be determined using any of these inhibitors interchangeably.

To further assess our probe in a cellular context, [³H]**1** binding in HEK293T P301S hTau cells overexpressing GSK-3 α/β , shRNA-induced GSK-3 α/β knockdown, and non-overexpressing HEK293T P301S hTau cells was compared (Figures 1H–J and S1F). Significantly higher binding was detected in overexpressing cells compared to GSK-3 α/β knockdown (three-fold) and control (six-fold) while cold chase studies (where

nonradioactive agent is administered following the radiotracer) resulted in consistent time-dependent binding reductions in all cells (Figure 1H,I). Strong, dose-dependent, GSK-3 specific, and reversible binding was observed with overexpressing HEK293T P301S hTau cells when incubated with [³H]**1** in competition (co-incubation, 85% displacement at 5 μ M) and pulse chase (96% displacement at 5 μ M for 5 min) studies with a third heterologous inhibitor (e.g., AR79, Figure S1A,G). Further evidence of GSK-3 α/β target engagement and selectivity on brain tissue was found when comparing tumor to normal CNS contralateral tissue binding of [³H]**1** in rat brain sections with intracranial GS9L gliomas in vitro (>40% binding differences, Figure S1D,E). We next performed binding saturation experiments using mouse brain homogenates which determined a GSK-3 α/β binding site density ($B_{\max}$) of 960.33 ± 234 fmol/mg and an affinity in line with biochemical data ($K_D = 13.02 \pm 3.3$ nM) (Figure 1G). Consistently throughout these cellular and whole tissue assays, the observed signals are predictably derived from GSK-3 α and GSK-3 β —as large-scale proteomic analyses have indicated only about two-to fourfold higher CNS $B_{\max}$ for GSK-3 β when compared to GSK-3 α .^{33,34} Together, these results also indicated that the **1** scaffold aligns suitable target engagement and selectivity properties to serve as a starting point in our lead-to-candidate optimization efforts.

Development and Characterization of Selective Oxazole-4-carboxamide GSK-3 Inhibitors.

The hit-to-lead screening campaign which led to **1** had pointed out the importance of three key fragments with regard to GSK-3 potency and selectivity: (1) the 1,2,4-triazol-1-yl group involved in a cation- π interaction with Arg141; (2) a critical core oxazole-4-carboxamide moiety providing hinge domain–ligand hydrogen bond-pairing interactions around Val135/Asp133; and (3) the 3-chloro-4-methoxyphenyl ring mainly involved in hydrophobic contacts within the deep pocket formed between the N- and C-lobes in the vicinity of Phe67, Lys85, and Leu132 (GSK-3 β residues) and corresponding GSK-3 α residues.²⁸ On the basis of the aforementioned in vitro cell and tissue studies, and guided by structural data gained previously, we undertook structural refinement efforts to improve blood–brain barrier (BBB) permeation, which is suboptimal for **1**,²⁸ and in vivo properties of our lead while retaining or improving physical and biochemical properties.²⁸ To identify next-generation inhibitors, >50 optimized compounds were synthesized and underwent biochemical screening (Figure 2B, Supporting Information Materials and Methods and Tables S1–S4). In designing derivatives, emphasis was placed on the exploration of the effects of halogenation patterns, primarily fluorinations, and the modulation of the cation- π interacting group while keeping the oxazole-4-carboxamide pharmacophore intact and minimizing changes detrimental to calculated Fu_p (unbound brain fraction) and CNS multiparameter optimization (MPO) scores (properties which are suboptimal for most known GSK-3 inhibitors, including PET probes evaluated to date, Figure 2B, Supporting Information Materials and Methods and Tables S1–S4). Our structure–activity relationship (SAR) study around the cation- π interacting side chain amide substituents showed that 6-membered nitrogen-containing rings (pyrazine, pyridines) combined with 3-“chlorine-for-fluorine” substitutions at the 4-methoxyphenyl fragment were well tolerated. We identified three inhibitors with such structural patterns displaying single-digit nanomolar potencies toward GSK-3 β (namely, PF-618 (**28**), OCM-37 (**37**), and OCM-44 (**44**); $IC_{50} < 2.5$ nM) in our ADPGlo screening assay (Figure 2A). Gratifyingly, these structural modifications were associated with

profound CNS-penetration enhancements in vivo (vide infra). The high binding affinity and dual GSK-3 α/β inhibitory nature of these analogues were confirmed in a second biochemical assay format, utilizing radiometric filtration (γ -[³³P]-ATP kinase assay, Supporting Information Materials and Methods) (GSK-3 α/β K_i = 3.3/4.2 nM, 28, Figure 2C,D). When assayed against a comprehensive panel of 371 kinase targets, these inhibitors displayed distinctive selectivity, inhibiting only two targets (DYRK1A and DYRK1B) beyond IC₅₀ concentrations at 1.0 μ M (Figure 2F, Tables S5 and S6). While activity overlap between GSK-3 and the Down syndrome kinases DYRK1s is common across inhibitors aimed at both targets, we observed that in this case, it may be exacerbated by ligand-receptor π - π interactions from a conserved DYRK1A/B Asp247/198, mimicking the guanidinium Arg141 cation- π interaction (Figure 2E,G-I).³⁵ However, we found 40–170-fold selectivity for GSK-3 α/β when measuring specific inhibitory constants even against the closest off-targets (DYRK1A/DYRK1B K_i = 325 nM/171 nM; Figure 2C,D), well within the requirements expected for therapeutics and PET radioligand development.

Oxazole-4-carboxamide Probes Engage npS9-GSK-3 β and pS9-GSK-3 β Irrespective of Activation States.

Pathological changes associated with GSK-3 β may be overexpression- or overactivation-driven, therefore it is crucial to address whether molecular probes engaging GSK-3 β do so dependently or independently of the S9 phosphorylation status in order to extrapolate valid imaging applications (S21 in GSK-3 α isozyme). To elucidate possible target engagement discrepancies between npS9-GSK-3 β (active nonphospho-S9) and pS9-GSK-3 β (inactive phospho-S9), we developed a high-sensitivity microscale thermophoresis (MST)-based binding assay. Use of MST allows for high-precision determination of equilibrium dissociation constants by measuring temperature-induced changes in a fluorescently labeled target protein as a function of small amounts of nonfluorescent small molecules with minimal influence by ATP concentration and other such factors that impact functional cell-based assays.³⁶ We generated catalytically inactive pS9-GSK-3 β by treating nonphosphorylation active npS9-GSK-3 β (Figure 3B) with AKT and ATP. When npS9-GSK-3 β was treated with AKT and high ATP concentration for 3 h, complete loss of catalytic activity was observed, and high level of pS9-GSK-3 β was produced as determined by ADPGlo kinase and western blot analyses (Figure 3C,D). Under control conditions using ATP treatments alone, GSK-3 β catalytic activity remained unaffected (Figure 3C,D). Characterization by MST of the npS9-GSK-3 β and pS9-GSK-3 β proteins first confirmed the high binding affinities of oxazole-4-carboxamide inhibitors toward npS9-GSK-3 β (K_d = 0.8 and 4.5 nM for **1** and **44** respectively) and revealed only marginal differences versus inactive pS9-GSK-3 β with regard to target engagement (<two-fold, Figure 3E-I). Under the same conditions, CHIR99021 GSK-3 β binding was also largely independent of the S9 status (K_d = 48.8 and 28.3 nM for pS9-GSK-3 β and npS9-GSK-3 β , respectively, Figure S2A).

Consistent with these MST results, in silico experiments and X-ray crystal structure analyses indicated that conformational changes occurring upon N-terminal inhibitory peptide sequence binding do so remotely from the inhibitor-binding site (Figure 3A). GSK-3 β structural remodeling following pS9 autoinhibition primarily affects the C-loop and activation segment, which are positioned distantly from the bound inhibitor and have

negligible impact on the ATP-binding site conformations, thus having limited impact on the target engagement of type-I ATP competitive inhibitors as observed here (Figure 3A).³⁷ On the basis of these results, we conclude that oxazole-4-carboxamide-based probes, such as [³H]**1** and positron emitter-labeled derivatives thereof, provide a genuine assessment of total GSK-3 density irrespective of the S9 phosphorylation.

Discovery of Structural Determinants To Generate Picomolar GSK-3 β -Isozyme Selective Inhibitors.

In the course of the initial SAR study, we observed that the orientation of the 3-halogen phenyl substituents found in this series was optimal with respect to the deep hydrophobic cleft found in GSK-3 β (Figures 2A,H and C, E–G). Close inspection of GSK-3 β -**1** and other GSK-3 β cocrystal structures indicated that, as recently noted by Wagner et al. 2018, the topology of this pocket is dependent on a complex backend hydrogen-bonding network involving the side chain of Asp133, the only nonconserved residue from the ATP-binding site between GSK-3 α and GSK-3 β (Glu196 in GSK-3 α).³² We observed that the hydrogen bond interactions in GSK-3 β which bring the side chains of Asp133, Arg113, and Glu80 in close contact also directly impact proximal hydrophobic residues from β sheets 5 and 6 (gatekeeper Leu132, Leu130 and Leu112), pulling these residues away from the ATP-binding site and forcing the opening a deep yet narrow hydrophobic pocket, wherein the paralogue GSK-3 α residue Glu196 could lack the ability to secure such interactions in the first place. Finer tuning of the occupation of this pocket using subtle variation in halogen patterning, we reasoned, would allow for the possibility of achieving β -isozyme selectivity at high potency.

To assess this, we developed a subseries of derivatives leading to inhibitor **51**, which gratifyingly provided not only 65-fold improvement in potency compared to **44** (GSK-3 β IC₅₀ = 0.031 nM) but also >10-fold selectivity for GSK-3 β over GSK-3 α (Figures 4A,C,E–G and S2B). Similar results were obtained using our confirmation [³³P]-radiometric binding assays (*K*_i = 0.10 nM, >10-fold GSK-3 β selectivity over GSK-3 α , Figure 4B,D). Increases in potency moving from **44** to **1** (via inhibitor OCM-47 (**47**)/49 (**49**)) could be attributed to both the incorporation of gradually bulky aryl halides substituents (F < Cl < I) and the use of an optimized 5-fluoropyridin-3-yl cation- π interacting moiety (Figure 4A,D). However, the β -isozyme selectivity occurred specifically with the iodoaryl substitution (Figure 4E–G). We confirmed this effect in a similar subseries wherein the cation- π interacting fragment was kept untouched ([PF-618 (**28**), OCM-45 (**45**) and OCM-32 (**32**) Figure 4A,D, Tables S3 and S4]. As for previous compounds of this series, these inhibitors displayed exquisite selectivity in broad kinome coverages (Figure 4I). Inhibitor **51** demonstrated improved selectivity even compared to previous inhibitors from this series (vide supra), with 10000-fold selectivity against all 371 kinases tested including the DYRK family. Further analysis of the potent inhibitor **49** as a representative example of this series in Kinativ proteomic screening using mouse brain lysate revealed extensive GSK-3 α / β kinase engagement without any detectable off-target interactions (>215 kinases, Figure 4H). Notably, the Kinativ profiling used here included >40 CNS-enriched kinases not initially covered in recombinant enzyme panels. We obtained dissociation constants from MST analysis in line with biochemical data and showed, as previously noted, no quantifiable affinity differences between npS9-GSK-3 β and

pS9-GSK-3 β enzymes (**51** K_d = 0.39 nM and K_d = 0.49 nM for npS9-GSK-3 β and pS9-GSK-3 β , respectively; Figures 4L,M and S3A). Employing a high concentration cutoff of 10 μ M, we also found negligible off-target binding for the representative inhibitor **51** in a CNS-related panel, with only 5 of 80 proteins (including receptors, ion channels and transporters), exhibiting >70% inhibition at this concentration (Figure 4J). Follow-up inhibitory constant measurements revealed >1000–20000-fold gaps in favor of GSK-3 β in all cases providing further proof of selectivity (Figure 4K). In addition, all representative compounds tested (**28**, **44**, and **51**) display low hERG inhibition (EC_{50} = 1.7–62.6 μ M) and correspondingly significant in vitro margins with regard to GSK-3 β inhibition (8320–56667-fold, Figure S2C).

Bioactivities of the Oxazole-4-carboxamide Inhibitors in Cells.

We next explored the effect of oxazole-4-carboxamide inhibitors on endogenous cellular GSK-3 β signaling. To define the cellular effect on pTau, we used high-content imaging of human cortical-like glutamatergic neurons differentiated from induced pluripotent stem cells through induced expression of Ngn2 transgene.^{18,38} In agreement with high biochemical potency and cellular target engagement, we found that the two novel representative compounds tested (**28** and **51**) caused dose-dependent decreases in pTau levels with efficacy similar to **1** (Figures 5A–D and S3C). We employed as the negative control the low potency, trifluoromethoxy-bearing structural analogue OCM-29 (**29**; IC_{50} = 4.1 μ M, Figure S3A) and observed no appreciable decrease in pTau levels even with doses up to 30 μ M (Figure 5D). We further evaluated the oxazole-4-carboxamide inhibitors in another neuronal assay, in which the phosphorylation of collapsin response mediator protein 2 (CRMP2) at Thr-514 (pCRMP2^{T514}) is measured using immunostaining, following 24 h treatment with test compounds.³⁹ CRMP2 is a direct protein substrate of GSK-3 β , not GSK-3 α , and important for diverse neuronal functions.^{40,41} Compounds were tested at six doses from 20 μ M with 2-fold dilution. Robust dose-dependent effect with EC_{50} ranging from 0.5 to 1.5 μ M was detected for all active compounds, as well as the CHIR99021 control (Figures 5E–I and S3E,H). In contrast, the low potency, negative control **29** failed to elicit detectable pCRMP2^{T514} reductions even at the highest concentration tested (20 μ M, Figure 5H).

Given the role of GSK-3 in WNT/ β -catenin signaling, we also conducted Wnt-signaling gene transcription luciferase reporter assay (Figure 5N).⁴³ Relative to pTau and pCRMP2^{T514} inhibition, significantly higher concentrations of active oxazole-4-carboxamide inhibitors were required to elicit gene transcription through the WNT/ β -catenin pathway (>10–25-fold selectivity for pCRMP2^{T514} and 3–8-fold selectivity for pTau). Although 10 μ M CHIR99021 was sufficient to induce 10-fold changes in reporter induction (Figure S3D), oxazole-4-carboxamide inhibitor treatment only triggered two- to threefold changes under the same conditions. GSK-3 signaling is also essential for coordinating the proliferation and differentiation of progenitor cells during brain development.^{2,42} We used human neural progenitor cells (NPCs) and a proliferation assay to evaluate the proneurogenic potential of these GSK-3 inhibitors. In line with previous results, effect on cell proliferation was, however, minor and observed only at micromolar concentrations for all active inhibitors (Figures 5K,N and S3I,L). Together, these data indicate that these oxazole-4-carboxamide inhibitors can access endogenous GSK-3 in human neuronal cells

and modulate functions of various GSK-3 complexes with differential efficacies, delineating a potential therapeutic window wherein the inhibition of GSK-3 β leads to the modulation of key neuronal molecular targets while sparing putative mechanism-based β -catenin-driven effects.

Oxazole-4-carboxamide Probes Display Increased Specific Binding in Tg2576 Mice Brains in Vitro and Robust BBB Permeation in Vivo.

Given the implication of GSK-3 α/β in APP processing and AD pathophysiology, we sought to determine in vitro GSK-3 α/β -targeted radioligand binding in the brain tissue from transgenic mice with significant amyloid plaque burden. Remarkably, comparison between Tg2576 mice (15 month) and old wild-type animals (21 and 28 months) revealed significant differences in [³H]**1** total and specific binding in the brain tissue (Figure 6A). As previously, we carried out triple-blocking experiments (10 μ M of **1**, CHIR99021, or LY2090314) to confirm specificity and selectivity (80–91% blocking) in these tissues (Figure 6A). Although we observed a suitable total and specific binding level correlation between old control mice and other normal brain tissue from rats, marmosets, and humans (vide supra), we discovered important increases in total/specific binding in Tg2576 mice compared to 21-month controls. Binding changes were drastic and most pronounced in regions known to exhibit high amyloid plaque deposition such as the cortex (17-fold increase) while binding in regions which present with lower or negligible plaque burden such as the hippocampus (six-fold increase) or the caudate putamen (four-fold increase), respectively, were significantly less affected (Figure 6C).^{44,45} In addition, regional distribution aligned well with previously characterized ubiquitous tracer binding in the brains of all other species (Ctx > HP > CB > CPu > WM) (Figure 1).

With the dual goals of identifying putative GSK-3-targeted therapeutics for CNS diseases and defining structural basis to achieve PET neuroimaging for this target, we coupled the initial SAR component of this study with iterative micro-dosing rodent PET imaging experiments to gain insight into structural factors associated with BBB permeation. As referred to previously (vide supra), this led to the early realization that six-membered nitrogen-containing replacements of the triazole ring found in **1**, while upholding or improving other properties from our lead, triggered significant increases in brain exposure. Among selected inhibitors ([¹¹C]PF-627 (**20**), [¹¹C]**28**, [¹¹C]**37**, [¹¹C]**44**, and [¹¹C]**51**, Supporting Information Materials and Methods Scheme S5 and Figures S5–S21), uptake of radioactivity in the rat brain following microdosed peripheral injection (total dose = 0.19–1.75 μ g i.v.) was variable but in all cases non-negligible with whole brain SUV_{peak} ranging from 0.84 to 3.08 followed by rapid washout (Figure S4A). We observed the highest BBB permeation with [¹¹C]**28** and [¹¹C]**44** while the lowest radioactivity brain uptake was seen with the most potent inhibitor, [¹¹C]**51**, which may indicate substantial GSK-3 binding in the periphery prior to reaching the CNS. Alternatively, this may be because of a faster clearance from the blood. Consistently, regional brain radioactivity following administration was largely homogeneous as observed in vitro. Next, to measure brain kinetics and determine brain exposure in higher species as a mean to assess the potential of our inhibitors as therapeutics and radioligands, we performed micro-dosed PET imaging experiments in nonhuman primates (one female rhesus macaque, total dose = 1.1 μ g i.v.). The selected

radiolabeled inhibitors, [^{11}C]28, [^{11}C]37, and [^{11}C]44, displayed in rhesus monkey both significant brain exposure with a SUV_{peak} of 3.0–4.0 SUV and favorable brain kinetic for imaging and CNS-targeted therapy following injection (Figures 2B,D and S4B,C). Radioactivity uptake was equally high in all gray matter regions but lower in the white matter. These results are in contrast with the in vivo profile of [^{11}C]1, previously reported and retested here, which demonstrate only low brain uptake in rhesus macaques (Figure 6B,E).²⁸ Finally, we conducted neuropharmacokinetic studies with our lead 44 to assess brain exposure over time. Conforming with microdosing data, we noted rapid ($T_{\text{max}} = 1.0$ h) excellent brain exposure (unbound $C_{\text{max}} = 941$ nM), following single oral administration in mice (50 mg/kg p.o.; Figure 6F). The unbound brain-to-plasma ratio ($K_{\text{p,uu}}$, unbound AUC_{last} ratios) was 1.06 and largely consistent between animals, indicating continued distribution equilibrium between brain and blood compartments and no efflux liability (Figure 6F). No clinical sign following oral administration of 44 at therapeutic dose in mice or micro-dosing in nonhuman primates for imaging was observed, and both procedures were well tolerated.

DISCUSSION AND CONCLUSIONS

Modulating molecular targets efficiently and with high accuracy, particularly in the brain, where the BBB severely limits the capacity of peripherally administered small molecules to achieve target engagement, is a significant challenge in CNS drug discovery. To date, the roles of GSK-3 α/β in neurodevelopmental and neurodegenerative diseases have been difficult to probe pharmacologically, owing to the complex nature of GSK-3 α/β -mediated cellular pathways and the lack of suitable inhibitors. The data presented here describe brain-penetrant selective inhibitors, which potently engage and inhibit GSK-3 in the mammalian brain tissue, including human brains, and modulate GSK-3-regulated neuronal pathways in cells. We also define the structural underpinning for achieving picomolar-selective GSK-3 β inhibition as well as β -isozymespecificity, exploiting discrete topological variations from the deep ATP-binding site of GSK-3 isozymes as previously noted by Wagner et al. 2018.³² While exhibiting GSK-3 β selectivity in ranges described for the few previously known GSK-3 isozyme selective inhibitors, the compounds described here do so at over two orders of magnitude higher potencies—which may be crucial in achieving a favorable therapeutic window for this target.^{26,32} Whether the maintenance of about the same fold-differences in isozyme selectivity observed here with double-digit picomolar versus previously described double-digit nanomolar potency inhibitors represents an intrinsic limitation ensuing from GSK-3 α/β binding sites dissimilarities, or isozyme selectivity can be improved further, will require further detailed SAR work based on the results presented here. Previous studies have shown that deletion in embryonic stem cells of GSK-3 α or GSK-3 β do not individually alter β -catenin/TCF-mediated transcription, and that isoform-selective pharmacological inhibition of GSK-3 α in acute myeloid leukemia cells prevents Wnt/ β -catenin changes at specific concentrations where dual inhibition lacks such ability—thus providing strong rationale for isozyme-selective inhibition in mitigating putative mechanism-based toxicity driven by β -catenin translocation.^{9,32} In the current study, the lack of dual inhibitors equipotent to 51 (or 32) has impeded the determination of such effect directly. It is important to note however, that inhibitors 51 and 32 only trigger Wnt/ β -catenin transcriptional changes at higher

concentrations than dual and drastically lower potency controls (**1** and CHIR99021). This suggests that the GSK-3 β -isozyme selective inhibitors identified here may in fact cause similar shifts wherein GSK-3 β inhibition is achieved while sparing Wnt/ β -catenin. Irrespective of this effect, we note for all inhibitors identified here, a significant and favorable gap between the doses required to trigger Wnt/ β -catenin signaling changes and modulate p-CRMP2^{T514} and pTau levels.

Kinase-targeted in vivo noninvasive neuroimaging to explore intractable neurodegenerative and psychiatric conditions constitutes an unmet objective despite tremendous progress in the therapeutic and imaging use of kinase inhibitors outside the CNS.^{46,47} Our preclinical data demonstrate that radiolabeled oxazole-4-carboxamide inhibitors such as [¹¹C]**28** and [¹¹C]**44** align suitable physicochemical parameters, kinetics, and in vitro/in vivo properties to serve as leads to achieve GSK-3 PET imaging.⁴⁸ Our analysis of GSK-3 α/β brain density further supports the view that this target can be imaged using PET. Findings indicating that oxazole-4-carboxamide probes provide total GSK-3 density readouts and display severe binding increases in AD mice compared to wild-type animals also provide a basis for studying the potential of these probes to measure changes in GSK-3 expression in neurodegeneration. A possible caveat pertaining to the characterization of the activation-independent binding mode of the (radio)ligands described here is the report of an alternative p38 MAPK-mediated GSK-3 β inactivation phosphorylation at the S389 C terminus residue.⁴⁸ The relevance of this inactivation pathway has, however, only been characterized in mice and may not be relevant in human. Based on the results presented here, detailed PET quantification studies are currently underway. The major finding of this work is the discovery of orally available inhibitors with unique pharmacological profiles showing robust GSK-3 target engagement in the cell and tissue as well as high brain exposure, which warrants future intervention and imaging investigations.

EXPERIMENTAL SECTION

Chemical Synthesis.

Compound **PF-04802367** (98%) (hereafter called **1**), CHIR99021 (98%), and LY2090314 (98%) were purchased from Millipore Sigma. Alternatively, **1** was synthesized as described previously.²⁸ Detailed synthetic procedures for all inhibitors are provided in Schemes S1–S3 and Supporting Information Materials and Methods. Briefly, the lead inhibitor (target compound), 5-(3-fluoro-4-methoxyphenyl)-*N*-(3-(pyridin-3-yl)propyl)oxazole-4-carboxamide (**44**, OCM-44), was synthesized as follows: the title compound was synthesized from 5-(3-fluoro-4-methoxyphenyl)-oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and 3-(pyridin-3-yl)propan-1-amine (0.137 g, 1.00 mmol) to give **44** (0.145 g, 48%) as an off-white solid. LCMS (ESI): *m/z* = 356.10 [M + H]⁺. HRMS (ESI⁺): *m/z* calcd for C₁₉H₁₉FN₃O₃⁺ (M + H)⁺, 356.1410; found, 356.1402. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.55 (s, 1H), 8.49 (t, *J* = 5.60 Hz, 1H), 8.45 (d, *J* = 2.00 Hz, 1H), 8.39 (dd, *J* = 1.40, 4.80 Hz, 1H), 8.35 (dd, *J* = 2.20, 13.20 Hz, 1H), 7.97 (d, *J* = 8.40 Hz, 1H), 7.66–7.64 (m, 1H), 7.32–7.28 (m, 2H), 3.91 (s, 3H), 3.34–3.26 (m, 2H), 2.63 (t, *J* = 7.80 Hz, 2H), 1.84 (quin, *J* = 7.34 Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 161.09, 160.65, 150.78 (d, *J* = 241.3 Hz), 149.80, 149.74, 148.92, 148.30 (d, *J* = 10.6 Hz), 136.38, 128.31, 124.44 (d, *J* = 3 Hz),

122.67, 121.15, 119.68 (d, $J = 8.1$ Hz), 115.49 (d, $J = 21.5$ Hz), 113.64, 56.08, 38.20, 30.38, 29.58. Purity: HPLC $t_R = 5.55$ min, HPLC purity > 95.4%.

All final compounds were determined to have purity > 95% by HPLC. A X-select CSH C18 (4.6 × 150 mm 3.5 μ m) HPLC column was used to determine the chemical purity. The radiosynthesis quality control was performed on a Phenomenex Luna C18, 5 mm, 4.6 × 150 mm column. Liquid chromatography mass spectroscopy utilized a X-select CSH C18 (3.0 × 50mm 2.5 μ m) column.

General Procedure for Synthesis of Inhibitors 6–37, 39–42, and 44–51 (OCM-6–OCM-37, OCM-39–OCM-42, and OCM-44–OCM-51).

To a mixture of an appropriate carboxylic acid (**74–84**, 1 equiv), the corresponding primary amine (1.2 equiv) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (1.2 equiv) in dimethylformamide (DMF; 10 vol) were added triethylamine (5 equiv) in dropwise manner. The reaction mixture was stirred at room temperature for 2 h. The progress of the reaction was monitored by thin-layer chromatography (50% EtOAc in hexane). After completion, the reaction mass was taken into water and extracted with EtOAc, combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum to get crude mass. Unless otherwise noted, purification by reverse phase column chromatography using acetonitrile–water as the mobile phase was used to obtain purified final products, which were then triturated with pentane, filtered, and dried under vacuum to afford **6–37**, **39–42**, and **44–51** (OCM-6–OCM-37, OCM-39–OCM-42 and OCM-44–OCM-51).

N-(3-(1*H*-1,2,4-Triazol-1-yl)propyl)-5-(3,4-difluorophenyl)-oxazole-4-carboxamide (6**, OCM-6).—**The title compound was synthesized from 5-(3,4-difluorophenyl)oxazole-4-carboxylic acid (0.15 g, 0.66 mmol) and 3-(1*H*-1,2,4-triazol-1-yl)propan-1-amine (0.10 g, 0.80 mmol) to give **6** (0.12 g, 51%) as an off-white solid. LCMS (ESI): $m/z = 334.10$ [$M + H$]⁺. HRMS (ESI⁺): m/z calcd for C₁₅H₁₄F₂N₅O₂⁺ ($M + H$)⁺, 334.1116; found, 334.1112. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.66–8.64 (m, 2H), 8.56–8.50 (m, 2H), 8.04–8.02 (m, 1H), 7.96 (s, 1H), 7.63–7.56 (m, 1H), 4.22 (t, $J = 6.80$ Hz, 2H), 3.27 (q, $J = 6.40$ Hz, 2H), 2.04 (quin, $J = 6.80$ Hz, 2H).

¹³C NMR (100 MHz, DMSO-*d*₆): δ 160.62, 151.37, 150.43 (m), 148.96 (m), 148.76 (m), 147.83 (m), 144.05, 129.36, 124.93 (m), 124.3 (dd, $J = 11$ and 3.48 Hz), 117.83 (d, $J = 17.3$ Hz), 117.27 (d, $J = 20.6$ Hz), 46.52, 36.00, 29.39. Purity: HPLC $t_R = 7.17$ min, HPLC purity > 99.7%.

N-(3-(1*H*-1,2,4-Triazol-1-yl)propyl)-5-(perfluorophenyl)oxazole-4-carboxamide (7**, OCM-7).—**The title compound was synthesized from 5-(perfluorophenyl)oxazole-4-carboxylic acid (0.4 g, 1.43 mmol) and 3-(1*H*-1,2,4-triazol-1-yl)propan-1-amine (0.108 g, 0.85 mmol) to give **7** (0.06 g, 11%) as a pink-colored solid after SFC purification. LCMS (ESI): $m/z = 388.4$ [$M + H$]⁺. HRMS (ESI⁺): m/z calcd for C₁₅H₁₁F₅N₅O₂⁺ ($M + H$)⁺, 388.0833; found, 388.0825. ¹H NMR (400 MHz, chloroform-*d*): δ 8.14 (s, 1H), 8.05 (s, 1H), 7.96 (s, 1H), 7.34 (br s, 1H), 4.28 (t, $J = 6.40$ Hz, 2H), 3.47 (q, $J = 6.8$ Hz, 2H), 2.20 (quin, $J = 6.80$ Hz, 2H).

^{13}C NMR not assigned. Purity: HPLC t_R = 7.21 min, HPLC purity > 96.5%.

N-(3-(1H-1,2,4-Triazol-1-yl)propyl)-5-(3,4,5-trifluorophenyl)-oxazole-4-carboxamide (8, OCM-8).—The title compound was synthesized from 5-(3,4,5-trifluorophenyl)oxazole-4-carboxylic acid (0.1 g, 0.41 mmol) and 3-(1H-1,2,4-triazol-1-yl)propan-1-amine (0.06 g, 0.48 mmol) to give **8** (0.058 g, 41%) as an off-white solid. LCMS (ESI): m/z = 352.10 $[\text{M} + \text{H}]^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{15}\text{H}_{13}\text{F}_3\text{N}_5\text{O}_2^+$ ($\text{M} + \text{H}$) $^+$, 352.1021; found, 352.1020. ^1H NMR (400 MHz, chloroform- d): δ 8.19–8.14 (m, 3H), 7.95 (s, 1H), 7.85 (s, 1H), 7.53 (br s, 1H), 4.28 (t, J = 6.40 Hz, 2H), 3.49 (q, J = 6.40 Hz, 2H), 2.21 (quin, J = 6.80 Hz, 2H).

^{13}C NMR (100 MHz, CDCl_3): δ 160.96, 152.18 (m), 149.75 (m), 148.23 (m), 143.29, 141.96, 139.48 (d, J = 15.7 Hz), 129.65, 122.62 (m), 112.69 (m), 47.06, 36.35, 29.92. Purity: HPLC t_R = 7.55 min, HPLC purity > 99.6%.

N-(3-(1H-1,2,4-Triazol-1-yl)propyl)-5-(3-fluoro-4-(trifluoromethoxy)phenyl)oxazole-4-carboxamide (9, OCM-9).—The title compound was synthesized from 5-(3-fluoro-4-(trifluoromethoxy)phenyl)oxazole-4-carboxylic acid (0.15 g, 0.51 mmol) and 3-(1H-1,2,4-triazol-1-yl)propan-1-amine (0.09 g, 0.61 mmol) to give **9** (0.090 g, 44%) as an off-white solid. LCMS (ESI): m/z = 400.15 $[\text{M} + \text{H}]^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{16}\text{H}_{14}\text{F}_4\text{N}_5\text{O}_3^+$ ($\text{M} + \text{H}$) $^+$, 400.1033; found, 400.1026. ^1H NMR (400 MHz, DMSO- d_6): δ 8.69 (br s, 2H), 8.54 (br s, 2H), 8.11 (br s, 1H), 7.97 (br s, 1H), 7.74 (br s, 1H), 4.23 (br s, 2H), 3.30–3.21 (m, 2H), 2.05 (br s, 2H).

^{13}C NMR not assigned. Purity: HPLC t_R = 8.05 min, HPLC purity > 98.2%.

N-(3-(1H-1,2,4-Triazol-1-yl)propyl)-5-(3-fluoro-4-(trifluoromethyl)phenyl)oxazole-4-carboxamide (10, OCM-10).—The title compound was synthesized from 5-(3-fluoro-4-(trifluoromethyl)phenyl)oxazole-4-carboxylic acid (0.2 g, 0.73 mmol) and 3-(1H-1,2,4-triazol-1-yl)propan-1-amine (0.11 g, 0.87 mmol) to give **10** (0.09 g, 33%) as an off-white solid. LCMS (ESI): m/z = 384.15 $[\text{M} + \text{H}]^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{16}\text{H}_{14}\text{F}_4\text{N}_5\text{O}_2^+$ ($\text{M} + \text{H}$) $^+$, 384.1084; found, 384.1068. ^1H NMR (400 MHz, chloroform- d): δ 8.37 (d, J = 11.6 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.18 (s, 1H), 7.99 (s, 1H), 7.94 (s, 1H), 7.73–7.69 (m, 1H), 7.59 (br s, 1H), 4.32 (t, J = 6.80 Hz, 2H), 3.53 (q, J = 6.40 Hz, 2H), 2.25 (quin, J = 6.40 Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3): δ 160.85, 158.26, 152.20, 149.96, 148.75 (d, J = 2.9 Hz), 143.30, 132.32 (d, J = 9.3 Hz), 130.68, 127.29, 123.54 (m), 120.92, 119.30 (dd, J = 45.5 and 12.6 Hz), 116.60 (d, J = 24.5 Hz), 47.07, 36.39, 29.93. Purity: HPLC t_R = 7.90 min, HPLC purity > 99.4%.

N-(3-(1H-1,2,4-Triazol-1-yl)propyl)-5-(3,5-difluoro-4-methoxyphenyl)oxazole-4-carboxamide (11, OCM-11).—The title compound was synthesized from 5-(3,5-difluoro-4-methoxyphenyl)-oxazole-4-carboxylic acid (0.2 g, 0.78 mmol) and 3-(1H-1,2,4-triazol-1-yl)propan-1-amine (0.118 g, 0.93 mmol) to give **11** (0.09 g, 32%) as an off-white solid. LCMS (ESI): m/z = 386.25 $[\text{M} + \text{Na}]^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{16}\text{H}_{16}\text{F}_2\text{N}_5\text{O}_3^+$ ($\text{M} + \text{H}$) $^+$, 364.1221; found, 364.1214. ^1H NMR (400 MHz, DMSO- d_6): δ 8.65–8.69 (m, 1H), 8.64 (s, 1H), 8.53 (s, 1H), 8.19–8.10 (m, 2H), 7.96 (s, 1H), 4.21 (t, J = 6.80 Hz, 2H),

4.01 (s, 3H), 3.27 (q, $J = 6.40$ Hz, 2H), 1.98–2.09 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 160.58, 154.39 (dd, $J = 251$ and 6.8 Hz), 151.39, 150.67, 150.40, 148.64, 144.07, 129.52, 121.59 (m), 112.08 (m), 61.76, 46.52, 36.03, 29.38. Purity: HPLC $t_R = 7.38$ min, HPLC purity > 96.6%.

N-(3-(1H-1,2,4-Triazol-1-yl)propyl)-5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxamide (12, OCM-12).—The title compound was synthesized from 5-(3-fluoro-4-methoxyphenyl)-oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and 3-(1H-1,2,4-triazol-1-yl)propan-1-amine (0.127 g, 1.00 mmol) to give **12** (0.09 g, 31%) as an off-white solid. LCMS (ESI): $m/z = 346.25$ $[\text{M} + \text{H}]^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{16}\text{H}_{17}\text{FN}_5\text{O}_3^+$ ($\text{M} + \text{H}$) $^+$, 346.1315; found, 346.1302. ^1H NMR (400 MHz, DMSO- d_6): δ 8.59–8.51 (m, 3H), 8.36–8.33 (m, 1H), 7.98 (s, 1H), 7.96 (s, 1H), 7.30 (t, $J = 8.80$ Hz, 1H), 4.22 (t, $J = 6.80$ Hz, 2H), 3.91 (s, 3H), 3.22–3.31 (m, 2H), 2.04 (quin, $J = 6.80$ Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 160.87, 151.39, 150.78 (d, $J = 241.3$ Hz), 149.99, 149.80, 148.36 (d, $J = 10.6$ Hz), 144.04, 128.15, 124.51, 119.63 (d, $J = 7.6$ Hz), 115.53 (d, $J = 21.5$ Hz), 113.65, 56.13, 46.54, 35.96, 29.48. Purity: HPLC $t_R = 6.95$ min, HPLC purity > 96.7%.

(Z)-5-(3-Fluoro-4-methoxyphenyl)-N-(3-phenylallyl)oxazole-4-carboxamide (13, OCM-13).—The title compound was synthesized from 5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and (Z)-3-phenylprop-2-en-1-amine (0.134 g, 1.01 mmol) to get **13** (0.040 g, 13%) as an off-white solid. LCMS (ESI): $m/z = 353.15$ $[\text{M} + \text{H}]^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{20}\text{H}_{18}\text{FN}_2\text{O}_3^+$ ($\text{M} + \text{H}$) $^+$, 353.1301; found, 353.1294. ^1H NMR (400 MHz, CDCl_3): δ 8.21–8.18 (m, 2H), 7.81 (s, 1H), 7.40–7.28 (m, 5H), 7.04 (t, $J = 8.8$ Hz, 1H), 6.65 (d, $J = 11.6$ Hz, 1H), 5.83–5.77 (m, 1H), 4.38 (t, $J = 6$ Hz, 2H), 3.96 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 160.95, 151.84 (d, $J = 243.8$ Hz), 151.73, 149.12 (d, $J = 10.6$ Hz), 147.48 (d, $J = 4.6$ Hz), 136.29, 131.85, 128.76, 128.36, 128.03, 127.75, 127.29, 124.91, 119.90 (d, $J = 7.6$ Hz), 116.04 (d, $J = 22$ Hz), 112.83, 56.22, 37.68. Purity: HPLC $t_R = 8.99$ min, HPLC purity > 97.0%.

N-Cinnamyl-5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxamide (14, OCM-14).—The title compound was synthesized from 5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and (E)-3-phenylprop-2-en-1-amine (0.134 g, 1.01 mmol) to get **14** (0.065 g, 21%) as an off-white solid. LCMS (ESI): $m/z = 353.20$ $[\text{M} + \text{H}]^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{20}\text{H}_{18}\text{FN}_2\text{O}_3^+$ ($\text{M} + \text{H}$) $^+$, 353.1301; found, 353.1287. ^1H NMR (400 MHz, CDCl_3): δ 8.22–8.18 (m, 2H), 7.82 (s, 1H), 7.48–7.23 (m, 6H), 7.05 (t, $J = 8.4$ Hz, 1H), 6.63 (d, $J = 16$ Hz, 1H), 6.34–6.27 (m, 1H), 4.25 (t, $J = 6$ Hz, 2H), 3.96 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 160.92, 151.87 (d, $J = 243.9$ Hz), 151.81, 149.15 (d, $J = 10.5$ Hz), 147.50 (m), 136.55, 132.26, 128.56, 128.09, 127.70, 126.41, 125.32, 124.97 (d, $J = 3.4$ Hz), 119.93 (d, $J = 7.6$ Hz), 116.08 (d, $J = 19.5$ Hz), 112.86, 56.24, 41.26. Purity: HPLC $t_R = 9.31$ min, HPLC purity > 99.4%.

5-(3-Fluoro-4-methoxyphenyl)-N-phenethyl-oxazole-4-carboxamide (15, OCM-15).—The title compound was synthesized from 5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.1 g, 0.42 mmol) and (2-phenylethan-1-amine (0.061 g, 0.504 mmol) to get **15** (0.110 g, 76%) as an off-white solid. LCMS (ESI): $m/z =$

341.10 [M + H]⁺. HRMS (ESI⁺): *m/z* calcd for C₁₉H₁₈FN₂O₃⁺ (M + H)⁺, 341.1301; found, 341.1295. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.53 (s, 1H), 8.44–8.41 (m, 1H), 8.33 (dd, *J* = 1.6 & 13.2 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.32–7.18 (m, 6H), 3.91 (s, 3H), 3.53–3.48 (m, 2H), 2.84 (t, *J* = 7.2 Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 160.57, 150.77 (d, *J* = 241.3 Hz), 149.88, 149.76, 148.33 (d, *J* = 10.5 Hz), 139.33, 128.59, 128.32, 128.18, 126.08, 124.44, 119.60 (d, *J* = 8 Hz), 115.47 (d, *J* = 21.5 Hz), 113.64, 56.09, 35.08. Purity: HPLC *t*_R = 9.26 min, HPLC purity > 99.6%.

5-(3-Fluoro-4-methoxyphenyl)-N-((1R,2R)-2-phenylcyclopropyl)-oxazole-4-carboxamide and 5-(3-Fluoro-4-methoxyphenyl)-N-((1S,2S)-2-phenylcyclopropyl)oxazole-4-carboxamide (16, OCM-16).—The title compound

was synthesized from 5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and *rac*-cis-2-phenylcyclopropan-1-amine (0.134 g, 1.01 mmol) to get **16** (0.110 g, 36%) as an off-white solid. LCMS (ESI): *m/z* = 353.10 [M + H]⁺. HRMS (ESI⁺): *m/z* calcd for C₂₀H₁₈FN₂O₃⁺ (M + H)⁺, 353.1301; found, 353.1290. ¹H NMR (400 MHz, CDCl₃): δ 8.16–8.13 (m, 2H), 7.65 (s, 1H), 7.35–7.22 (m, 5H), 7.04–6.97 (m, 2H), 3.95 (s, 3H), 3.34–3.28 (m, 1H), 2.47–2.41 (m, 1H), 1.53–1.47 (m, 1H), 1.20–1.16 (m, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 162.39, 151.81 (*J* = 242 Hz), 151.38, 149.05 (d, *J* = 11 Hz), 147.33 (d, *J* = 5 Hz), 136.24, 128.69, 128.36, 127.86, 126.58, 124.80, 119.91 (d, *J* = 8 Hz), 116.01 (d, *J* = 20 Hz), 112.79, 56.19, 28.57, 21.85, 12.71. Purity: HPLC *t*_R = 8.96 min, HPLC purity > 99.8%.

5-(3-Fluoro-4-methoxyphenyl)-N-((1S,2R)-2-phenylcyclopropyl)-oxazole-4-carboxamide and 5-(3-Fluoro-4-methoxyphenyl)-N-((1R,2S)-2-phenylcyclopropyl)oxazole-4-carboxamide (17, OCM-17).—The title compound

was synthesized from 5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and *rac*-trans-2-phenylcyclopropan-1-amine (0.134 g, 1 mmol) to get **17** (0.100 g, 33%) as an off-white solid. LCMS (ESI): *m/z* = 353.00 [M + H]⁺. HRMS (ESI⁺): *m/z* calcd for C₂₀H₁₈FN₂O₃⁺ (M + H)⁺, 353.1301; found, 353.1297. ¹H NMR (400 MHz, CDCl₃): δ 8.25–8.17 (m, 2H), 7.81 (s, 1H), 7.47 (s, 1H), 7.33–7.29 (m, 2H), 7.23–7.20 (m, 3H), 7.05 (t, *J* = 2.2 Hz, 1H), 3.96 (s, 3H), 3.15–3.10 (m, 1H), 2.24–2.19 (m, 1H), 1.40–1.31 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 162.16, 151.86 (d, *J* = 244.2 Hz), 151.77, 149.18 (d, *J* = 10.6 Hz), 147.43 (d, *J* = 4.3 Hz), 140.41, 128.41, 127.91, 126.42, 126.16, 125.01, 119.83 (d, *J* = 8.1 Hz), 116.02 (d, *J* = 21.5 Hz), 112.86, 56.23, 31.88, 24.70, 16.38. Purity: HPLC *t*_R = 9.31 min, HPLC purity > 99.9%.

5-(3-Fluoro-4-methoxyphenyl)-N-(3-phenylpropyl)oxazole-4-carboxamide (18, OCM-18).—The title compound was synthesized from 5-(3-fluoro-4-

methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) using commercial available amine 3-phenylpropan-1-amine (0.136 g, 1.01 mmol) to give **18** (0.09 g, 31%) as an off-white solid. LCMS (ESI): *m/z* = 355.10 [M + H]⁺. HRMS (ESI⁺): *m/z* calcd for C₂₀H₂₀FN₂O₃⁺ (M + H)⁺, 355.1458; found, 355.1453. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.54 (s, 1H), 8.47–8.44 (m, 1H), 8.36–8.32 (m, 1H), 7.96 (d, *J* = 8.8 Hz, 1H), 7.31–7.25 (m, 3H), 7.20–7.14 (m, 3H), 3.89 (s, 3H), 3.32–3.26 (m, 2H), 2.6 (t, *J* = 7.20 Hz, 2H), 1.86–1.78 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 161.09, 151.84 (d, *J* = 243.6 Hz), 151.58, 149.08

(d, $J = 10.9$ Hz), 147.45, 141.35, 128.44, 128.37, 128.21, 125.96, 124.93 (d, $J = 3.4$ Hz), 119.98 (d, $J = 7.5$ Hz), 116.06 (d, $J = 21.5$ Hz), 112.83, 56.21, 38.87, 33.27, 31.21. Purity: HPLC $t_R = 8.83$ min, HPLC purity > 99.4%.

5-(3-Fluoro-4-methoxyphenyl)-N-(3-(2-methyl-1H-imidazole-1-yl)propyl)oxazole-4-carboxamide (19, PF-981).—PF-981 was graciously provided by

Pfizer (HPLC purity > 95%). Physical state: white solid. HRMS (ESI⁺): m/z calcd for $C_{18}H_{20}FN_4O_3^+$ (M + H)⁺, 359.1519; found, 359.1514. ¹H NMR (400 MHz, CDCl₃): δ 8.14 (dt, $J = 2.1, 10.8$ Hz, 2H), 7.82 (s, 1H), 7.43 (t, $J = 5.8$ Hz, 1H), 7.12 (d, $J = 6.4$ Hz, 2H), 7.05 (t, $J = 8.5$ Hz, 1H), 4.05 (t, $J = 7.1$ Hz, 2H), 3.95 (s, 3H), 3.51 (m, 2H), 2.62 (s, 3H), 2.12 (t, $J = 6.8$ Hz, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 161.8, 153.1–150.6 (d, $J = 245.5$ Hz), 152.1 (d, $J = 2.7$ Hz), 149.3 (d, $J = 10.8$ Hz), 147.7, 144.1, 127.5, 124.9 (d, $J = 3.6$ Hz), 122.3, 119.9, 119.5 (d, $J = 7.9$ Hz), 116.0 (d, $J = 21.6$ Hz), 112.9, 56.3, 44.5, 36.1, 30.7, 11.7.

5-(3-Fluoro-4-methoxyphenyl)-N-(3-(2-oxopyridin-1(2H)-yl)-propyl)oxazole-4-carboxamide (20, PF-627).—PF-627 was graciously provided by Pfizer (HPLC purity >

95%). Physical state: white solid. HRMS (ESI⁺): m/z calcd for $C_{19}H_{19}FN_3O_4^+$ (M + H)⁺, 372.1360; found, 372.1367. ¹H NMR (400 MHz, CDCl₃): δ 8.21 (dd, $J = 12.8, 2.1$ Hz, 1H), 8.12 (d, $J = 8.8$ Hz, 1H), 8.1 (s, 1H), 7.84 (s, 1H), 7.55 (d, $J = 7.6$ Hz, 1H), 7.49 (d, $J = 7.7$ Hz, 1H), 7.09–6.99 (m, 2H), 6.45 (t, $J = 6.3$ Hz, 1H), 4.17 (t, $J = 6.2$ Hz, 2H), 3.94 (s, 3H), 3.47 (m, 2H), 2.08 (t, $J = 6.1$ Hz, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 163.1, 161.5, 153.1–150.6 (d, $J = 245.0$ Hz), 151.5 (d, $J = 2.4$ Hz), 149.0 (d, $J = 10.8$ Hz), 147.8, 140.4, 137.7, 128.3, 124.8 (d, $J = 3.6$ Hz), 120.56, 120.1 (d, $J = 7.9$ Hz), 116.2 (d, $J = 21.7$ Hz), 112.8, 107.9, 56.2, 47.3, 35.4, 29.8.

5-(3-Fluoro-4-methoxyphenyl)-N-(3-(1-methyl-1H-pyrazol-4-yl)-propyl)oxazole-4-carboxamide (21, PF-024).—PF-024 was graciously provided by

Pfizer (HPLC purity > 95%). Physical state: white solid. HRMS (ESI⁺): m/z calcd for $C_{18}H_{20}FN_4O_3^+$ (M + H)⁺, 359.1519; found, 359.1530. ¹H NMR (400 MHz, CDCl₃): δ 8.20–8.13 (m, 2H), 7.79 (s, 1H), 7.40 (s, 1H), 7.31 (br s, 1H), 7.02 (t, $J = 8.5$ Hz, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.46 (m, 2H), 2.57 (t, $J = 7.5$ Hz, 2H), 1.89 (t, $J = 7.3$ Hz, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 161.1, 153.1–150.6 (d, $J = 245.1$ Hz), 151.6 (d, $J = 2.4$ Hz), 149.1 (d, $J = 10.8$ Hz), 147.5, 137.5, 129.0, 128.2, 124.9 (d, $J = 3.6$ Hz), 121.0, 119.9 (d, $J = 7.9$ Hz), 116.1 (d, $J = 21.7$ Hz), 112.8 (d, $J = 2.1$ Hz), 56.2, 38.7, 38.6, 30.8, 21.5.

5-(3-Fluoro-4-methoxyphenyl)-N-(3-(2-methyl-1H-imidazol-4-yl)propyl)oxazole-4-carboxamide (22, PF-760).—PF-760 was graciously provided by

Pfizer (HPLC purity > 95%). Physical state: White solid. HRMS (ESI⁺): m/z calcd for $C_{18}H_{20}FN_4O_3^+$ (M + H)⁺, 359.1519; found, 359.1514. ¹H NMR (400 MHz, CDCl₃): δ 8.20 (dd, $J = 12.9, 2.0$ Hz, 1H), 8.01 (d, $J = 8.6$ Hz, 1H), 7.84 (s, 1H), 7.62 (t, $J = 6.3$ Hz, 1H), 7.02 (t, $J = 8.6$ Hz, 1H), 6.87 (s, 1H), 3.94 (s, 3H), 3.50 (m, 2H), 2.71 (s, 3H), 1.98 (t, $J = 6.5$ Hz, 2H), 1.25 (s, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 162.3, 153.0–150.5 (d, $J = 245.1$ Hz), 152.0 (d, $J = 2.4$ Hz), 149.3 (d, $J = 10.7$ Hz), 147.8, 143.3, 132.2, 127.5, 124.8 (d, $J = 3.4$ Hz), 119.6 (d, $J = 8.0$ Hz), 116.3 (d, $J = 21.8$ Hz), 155.0, 112.9, 56.3, 38.0, 29.0, 21.5, 11.2.

5-(3-Fluoro-4-(trifluoromethyl)phenyl)-N-(3-(pyrazin-2-yl)propyl)oxazole-4-carboxamide (23, OCM-23).—The title compound was synthesized from 5-(3-fluoro-4-(trifluoromethyl)phenyl)oxazole-4-carboxylic acid (0.2 g, 0.72 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.12 g, 0.87 mmol) to give **23** (0.085 g, 30%) as an off-white solid. LCMS (ESI): m/z = 395.15 $[M + H]^+$. HRMS (ESI⁺): m/z calcd for C₁₈H₁₅F₄N₄O₄⁺ (M + H)⁺, 395.1131; found, 395.1138. ¹H NMR (400 MHz, chloroform-*d*): δ 8.52–8.50 (m, 2H), 8.42 (s, 1H), 8.38 (d, J = 12.00 Hz, 1H), 8.25 (d, J = 8.00 Hz, 1H), 7.91 (s, 1H), 7.68 (t, J = 7.60 Hz, 1H), 7.59 (br s, 1H), 3.55 (q, J = 6.80 Hz, 2H), 2.95 (t, J = 7.60 Hz, 2H), 2.13 (quin, J = 7.20 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 160.52, 159.52 (d, J = 253 Hz), 156.61, 149.80, 148.65, 144.61, 144.04, 142.43, 132.46 (d, J = 9.3 Hz), 130.68, 127.19, 123.54 (m), 120.92, 119.30 (dd, J = 45.5 and 12.6 Hz), 116.60 (d, J = 24.4 Hz), 38.83, 32.63, 28.67. Purity: HPLC t_R = 8.52 min, HPLC purity > 95.3%.

N-(3-(Pyrazin-2-yl)propyl)-5-(3,4,5-trifluorophenyl)oxazole-4-carboxamide (24, OCM-24).—The title compound was synthesized from 5-(3,4,5-trifluorophenyl)oxazole-4-carboxylic acid (0.1 g, 0.41 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.068 g, 0.49 mmol) to give **24** (0.042 g, 29%) as an off-white solid. LCMS (ESI): m/z = 363.15 $[M + H]^+$. HRMS (ESI⁺): m/z calcd for C₁₇H₁₄F₃N₄O₂⁺ (M + H)⁺, 369.1069; found, 369.1075. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.52–8.46 (m, 2H), 8.41 (br s, 1H), 8.20–8.15 (m, 2H), 7.84 (s, 1H), 7.55 (s, 1H), 3.53 (q, J = 6.8 Hz, 2H), 2.92 (t, J = 7.6 Hz, 2H), 2.14–2.07 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 160.66, 156.61, 152.25 (m), 149.74 (m), 148.15 (m), 144.62, 144.04, 142.44, 134.64 (dt, J = 252.5 and 16.0 Hz), 129.94, 122.78 (m), 112.71 (m), 38.81, 32.62, 28.70. Purity: HPLC t_R = 8.20 min, HPLC purity > 99.7%.

5-(3,4-Difluorophenyl)-N-(3-(pyrazin-2-yl)propyl)oxazole-4-carboxamide (25, OCM-25).—The title compound was synthesized from 5-(3,4-difluorophenyl)oxazole-4-carboxylic acid (0.15 g, 0.66 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.11 g, 0.80 mmol) to give **25** was obtained (0.1 g, 43%), as an off-white solid. LCMS (ESI): m/z = 345.30 $[M + H]^+$. HRMS (ESI⁺): m/z calcd for C₁₇H₁₅F₂N₄O₂⁺ (M + H)⁺, 345.1163; found, 345.1161. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.52–8.50 (m, 2H), 8.42 (d, J = 2.4 Hz, 1H), 8.39–8.34 (m, 1H), 8.16–8.13 (m, 1H), 7.85 (s, 1H), 7.60–7.50 (m, 1H), 7.30–7.20 (m, 1H), 3.58–3.53 (m, 2H), 2.95 (t, J = 7.60 Hz, 2H), 2.13 (quin, J = 7.20 Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 160.46, 156.78, 150.42, 150.20 (m), 148.96 (m), 147.83, 144.46, 143.98 (d, J = 13.1 Hz), 142.28, 129.47, 124.88 (m), 124.3 (dd, J = 11 and 3.48 Hz), 117.83 (d, J = 17.7 Hz), 117.24 (d, J = 20.3 Hz), 38.32, 32.05, 28.46. Purity: HPLC t_R = 7.79 min, HPLC purity > 99.0%.

N-(3-(Pyrazin-2-yl)propyl)-5-(3,4,5-trifluorophenyl)oxazole-4-carboxamide (26, OCM-26).—The title compound was synthesized from 5-(3,5-difluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.78 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.128 g, 0.93 mmol) to give **26** (0.070 g, 24%) as an off-white solid. LCMS (ESI): m/z = 375.15 $[M + H]^+$. HRMS (ESI⁺): m/z calcd for C₁₈H₁₇F₂N₄O₃⁺ (M + H)⁺, 375.1269; found, 375.1267. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.60 (s, 1H), 8.58–8.56 (m, 1H), 8.54–8.50 (m, 1H), 8.44–8.42 (m, 1H), 8.15–8.08 (m, 2H), 4.00 (s, 3H), 3.36–3.31 (m, 2H), 2.80 (t, J = 7.60 Hz, 2H), 1.98–1.91 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 160.41, 156.77,

154.38 (dd, $J = 244.9$ and 6.4 Hz), 150.34, 148.51, 144.45, 143.95, 142.27, 129.62, 121.66 (m), 112.02 (m), 61.78, 38.33, 32.04, 28.43. Purity: HPLC $t_R = 7.98$ min, HPLC purity > 95.3%.

5-(3-Fluoro-4-hydroxyphenyl)-N-(3-(pyrazin-2-yl)propyl)-oxazole-4-

carboxamide (27, PF-796).—PF-796 was graciously provided by Pfizer (HPLC purity > 95%). Physical state: white solid. HRMS (ESI⁺): m/z calcd for $C_{18}H_{20}FN_4O_3^+$ ($M + H$)⁺, 343.1206; found, 343.1206. ¹H NMR (400 MHz, CD₃OD): δ 8.74–8.66 (m, 1H), 8.55 (d, $J = 2.7$ Hz, 1H), 8.23–8.07 (m, 2H), 7.83 (d, $J = 6.6$ Hz, 1H), 6.99 (t, $J = 8.8$ Hz, 1H), 3.47 (t, $J = 6.8$ Hz, 2H), 3.01 (t, $J = 7.5$ Hz, 2H), 2.11 (t, $J = 7.6$ Hz, 2H). ¹³C NMR (100 MHz, CD₃OD): δ 162.2, 157.6, 151.8–149.9 (d, $J = 239.8$ Hz), 151.6 (d, $J = 2.8$ Hz), 148.7, 146.9, 143.5, 143.3, 140.7, 127.2, 124.4, 118.7 (d, $J = 7.7$ Hz), 117.2, 115.9 (d, $J = 22.1$ Hz), 38.2, 31.8, 28.4.

5-(3-Fluoro-4-methoxyphenyl)-N-(3-(pyrazin-2-yl)propyl)-oxazole-4-

carboxamide (28, OCM-28).—The title compound was synthesized from 5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.138 g, 1.00 mmol) to give **28** (0.060 g, 20%) as an off-white solid. LCMS (ESI): $m/z = 357.10$ [$M + H$]⁺. HRMS (ESI⁺): m/z calcd for $C_{18}H_{18}FN_4O_3^+$ ($M + H$)⁺, 357.1363; found, 357.1369. ¹H NMR (400 MHz, DMSO- d_6): δ 8.58 (s, 1H), 8.54 (s, 2H), 8.50 (t, $J = 6.00$ Hz, 1H), 8.45 (d, $J = 2.40$ Hz, 1H), 8.35 (dd, $J = 2.00, 13.60$ Hz, 1H), 7.96 (d, $J = 8.80$ Hz, 1H), 7.29 (t, $J = 8.80$ Hz, 1H), 3.91 (s, 3H), 3.35–3.30 (m, 2H), 2.81 (t, $J = 7.6$ Hz, 2H), 1.95 (quin, $J = 7.20$ Hz, 2H). ¹³C NMR (100 MHz, DMSO- d_6): δ 160.69, 156.86, 150.77 (d, $J = 241.3$ Hz), 149.84, 149.75, 148.31 (d, $J = 10.9$ Hz), 144.45, 143.96, 142.27, 128.25, 124.45, 119.66 (d, $J = 8$ Hz), 115.48 (d, $J = 21.5$ Hz), 113.65, 56.18, 38.26, 32.06, 28.51. Purity: HPLC $t_R = 7.53$ min, HPLC purity > 99.7%.

5-(3-Fluoro-4-(trifluoromethoxy)phenyl)-N-(3-(pyrazin-2-yl)-propyl)oxazole-4-

carboxamide (29, OCM-29).—The title compound was synthesized from 5-(3-fluoro-4-(trifluoromethoxy)phenyl)-oxazole-4-carboxylic acid (0.15 g, 0.51 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.08 g, 0.61 mmol) to give **29** (0.11 g, 52%) as an off-white solid. LCMS (ESI): $m/z = 411.20$ [$M + H$]⁺. HRMS (ESI⁺): m/z calcd for $C_{18}H_{15}F_4N_4O_3^+$ ($M + H$)⁺, 411.1080; found, 411.1077. ¹H NMR (400 MHz, chloroform- d): δ 8.53–8.51 (m, 2H), 8.43 (d, $J = 2.40$ Hz, 1H), 8.40 (dd, $J = 1.60, 11.60$ Hz, 1H), 8.19 (d, $J = 8.80$ Hz, 1H), 7.89 (s, 1H), 7.58 (br s, 1H), 7.40 (t, $J = 8.00$ Hz, 1H), 3.56 (q, $J = 7.2$ Hz, 2H), 2.96 (t, $J = 7.60$ Hz, 2H), 2.17–2.10 (m, 2H). ¹³C NMR (100 MHz, DMSO- d_6): δ 160.35, 156.78 (d, $J = 246.8$ Hz), 150.88, 148.40, 144.47, 143.98, 142.30, 135.76 (d, $J = 14.4$ Hz), 130.33, 128.01, 127.92, 124.81 (m), 124.09, 120.15 (d, $J = 210$ Hz), 117.05 (d, $J = 22.3$ Hz), 38.35, 32.06, 28.44. Purity: HPLC $t_R = 8.66$ min, HPLC purity > 98.9%.

5-(4-Cyano-3-fluorophenyl)-N-(3-(pyrazin-2-yl)propyl)oxazole-4-carboxamide

(30, OCM-30).—The title compound was synthesized from 5-(4-cyano-3-fluorophenyl)oxazole-4-carboxylic acid (0.15 g, 0.65 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.11 g, 0.78 mmol) to give **30** (0.07 g, 31%) as an off-white solid. LCMS (ESI): $m/z = 352.10$ [$M + H$]⁺. HRMS (ESI⁺): m/z calcd for $C_{18}H_{15}FN_5O_2^+$ ($M + H$)⁺, 352.1210;

found, 352.1206. ^1H NMR (400 MHz, chloroform-*d*): δ 8.53–8.43 (m, 4H), 8.32 (dd, J = 1.47, 8.31 Hz, 1H), 7.93 (s, 1H), 7.70 (dd, J = 6.85, 8.31 Hz, 1H), 7.63 (br s, 1H), 3.55 (q, J = 6.36 Hz, 2H), 2.94 (t, J = 7.58 Hz, 2H), 2.17–2.10 (m, 2H). ^{13}C NMR (100 MHz, chloroform-*d*): δ 162.9 (d, J = 257 Hz), 160.4, 156.5, 149.0, 148.9 (d, J = 3 Hz), 144.6, 144.1, 142.5, 133.4, 133.26, 131.7, 124.0 (d, J = 4 Hz), 116.0 (d, J = 24 Hz), 113.7, 102.1 (d, J = 15.6 Hz), 38.9, 32.6, 28.6. Purity: HPLC t_R = 7.53 min, HPLC purity > 99.4%.

5-(3-Cyano-4-fluorophenyl)-N-(3-(pyrazin-2-yl)propyl)oxazole-4-carboxamide (31, OCM-31).—The title compound was synthesized from 5-(3-cyano-4-

fluorophenyl)oxazole-4-carboxylic acid (0.15 g, 0.65 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.11 g, 0.78 mmol) to give **31** (0.045 g, 20%) as an off-white solid. LCMS (ESI) = 352.05 [M + H] $^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{18}\text{H}_{15}\text{FN}_5\text{O}_2^+$ (M + H) $^+$, 352.1210; found, 352.1216. ^1H NMR (400 MHz, chloroform-*d*): δ 8.77–8.73 (m, 1H), 8.65 (dd, J = 2.20, 6.11 Hz, 1H), 8.53–8.48 (m, 2H), 8.42 (d, J = 2.45 Hz, 1H), 7.89 (s, 1H), 7.60 (br s, 1H), 7.32 (t, J = 8.56 Hz, 1H), 3.55 (q, J = 6.85 Hz, 2H), 2.94 (t, J = 7.58 Hz, 2H), 2.17–2.09 (m, 2H). ^{13}C NMR (100 MHz, chloroform-*d*): δ 163.4 (d, J = 262.4 Hz), 160.6, 156.6, 149.5, 148.4 (d, J = 4 Hz), 144.6, 144.0, 142.5, 135.1 (d, J = 9 Hz), 133.4, 129.9, 124.4 (d, J = 3 Hz), 116.7 (d, J = 20 Hz), 113.4, 102.0 (d, J = 16 Hz), 38.8, 32.6, 28.7. Purity: HPLC t_R = 7.45 min, HPLC Purity > 99.7%.

5-(3-Iodo-4-methoxyphenyl)-N-(3-(pyrazin-2-yl)propyl)oxazole-4-carboxamide (32, OCM-32).—The title compound was synthesized from 5-(3-iodo-4-

methoxyphenyl)oxazole-4-carboxylic acid (0.35 g, 1.00 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.16 g, 1.20 mmol) to give **32** (0.18 g, 38%) as a pale yellow solid. LCMS (ESI) = 465.05 [M + H] $^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{18}\text{H}_{18}\text{IN}_4\text{O}_3^+$ (M + H) $^+$, 465.0424; found, 465.0410. ^1H NMR (400 MHz, chloroform-*d*): δ 8.64 (d, J = 1.96 Hz, 1H), 8.53–8.48 (m, 3H), 8.41 (d, J = 2.45 Hz, 1H), 7.80 (s, 1H), 7.48 (br s, 1H), 6.89 (d, J = 8.80 Hz, 1H), 3.94 (s, 3H), 3.54 (q, J = 6.85 Hz, 2H), 2.93 (t, J = 7.58 Hz, 2H), 2.15–2.06 (m, 2H). ^{13}C NMR (100 MHz, chloroform-*d*): δ 160.5, 158.6, 156.1, 150.6, 146.9, 143.9, 143.4, 141.7, 138.2, 129.7, 127.3, 120.8, 109.6, 84.8, 55.8, 38.0, 32.0, 28.2. Purity: HPLC t_R = 8.24 min, HPLC Purity > 98.8%.

5-(3-Fluoro-4-(trifluoromethyl)phenyl)-N-(3-(pyridin-2-yl)-propyl)oxazole-4-carboxamide (33, OCM-33).—The title compound was synthesized from 5-(3-fluoro-4-

(trifluoromethyl)phenyl)oxazole-4-carboxylic acid (0.2 g, 0.72 mmol) and 3-(pyridin-2-yl)propan-1-amine (0.12 g, 0.87 mmol) to give **33** (0.1 g, 35%) as an off-white solid. LCMS (ESI): m/z = 394.15 [M + H] $^+$. HRMS (ESI $^+$): m/z calcd for $\text{C}_{19}\text{H}_{16}\text{F}_4\text{N}_3\text{O}_2^+$ (M + H) $^+$, 394.1179; found, 394.1166. ^1H NMR (400 MHz, chloroform-*d*): δ 8.56 (d, J = 4.40 Hz, 1H), 8.39 (d, J = 12.00 Hz, 1H), 8.26 (d, J = 8.00 Hz, 1H), 7.92 (s, 1H), 7.76 (br s, 1H), 7.71 (t, J = 14.2 Hz, 1H), 7.60 (dt, J = 1.60, 7.60 Hz, 1H), 7.19 (d, J = 8.00 Hz, 1H), 7.15–7.12 (m, 1H), 3.53 (q, J = 6.50 Hz, 2H), 2.92 (t, J = 7.40 Hz, 2H), 2.11 (quin, J = 7.10 Hz, 2H). ^{13}C NMR not assigned. Purity: HPLC t_R = 6.27 min, HPLC purity > 96.5%.

N-(3-(Pyridin-2-yl)propyl)-5-(3,4,5-trifluorophenyl)oxazole-4-carboxamide (34, OCM-34).—The title compound was synthesized from 5-(3,4,5-trifluorophenyl)oxazole-4-

carboxylic acid (0.2 g, 0.82 mmol) and 3-(pyridin-2-yl)propan-1-amine (0.135 g, 1.00 mmol) to give **34** (0.030 g, 10%) as an off-white solid. LCMS (ESI): m/z = 362.10 [M + H]⁺. HRMS (ESI⁺): m/z calcd for C₁₈H₁₅F₃N₃O₂⁺ (M + H)⁺, 362.1116; found, 362.1109. ¹H NMR (400 MHz, chloroform-*d*): δ 8.56 (d, *J* = 4.4 Hz, 1H), 8.30–8.15 (m, 2H), 7.86 (s, 1H), 7.73 (br s, 1H), 7.64–7.60 (m, 1H), 7.21–7.19 (m, 1H), 7.15–7.12 (m, 1H), 3.53 (q, *J* = 6.50, 2H), 2.93 (t, *J* = 7.6 Hz, 2H), 2.11 (quin, *J* = 10.6 Hz, 2H) (one CH₂ merged in solvent peak). ¹³C NMR not assigned. Purity: HPLC *t*_R = 5.94 min, HPLC purity > 99.0%.

5-(3,4-Difluorophenyl)-N-(3-(pyridin-2-yl)propyl)oxazole-4-carboxamide (35, OCM-35).—The title compound was synthesized from 5-(3,4-difluorophenyl)oxazole-4-carboxylic acid (0.12 g, 0.53 mmol) and 3-(pyridin-2-yl)propan-1-amine (0.087 g, 0.64 mmol) to give **35** (0.09 g, 49%) as an off-white solid. LCMS (ESI): m/z = 344.05 [M + H]⁺. HRMS (ESI⁺): m/z calcd for C₁₈H₁₆F₂N₃O₂⁺ (M + H)⁺, 344.1211; found, 344.1214. ¹H NMR (400 MHz, chloroform-*d*): δ 8.56 (d, *J* = 3.2 Hz, 1H), 8.40–8.34 (m, 1H), 8.17–8.13 (m, 1H), 7.85 (s, 1H), 7.67 (br s, 1H), 7.60 (dt, *J* = 2.00, 7.80 Hz, 1H), 7.26–7.11 (m, 3H), 3.52 (q, *J* = 6.50 Hz, 2H), 2.93 (t, *J* = 7.40 Hz, 2H), 2.18–2.06 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 160.06, 160.42, 150.77 (m), 150.41, 148.91, 148.29 (m), 136.42, 129.53, 124.92 (m), 124.42 (m), 122.69, 121.17, 117.84 (d, *J* = 17.7 Hz), 117.24 (d, *J* = 20.7 Hz), 38.54, 35.01, 28.94. Purity: HPLC *t*_R = 5.69 min, HPLC purity > 96.6%.

5-(3,5-Difluoro-4-methoxyphenyl)-N-(3-(pyridin-2-yl)propyl)-oxazole-4-carboxamide (36, OCM-36).—The title compound was synthesized from 5-(3,5-difluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.140 g, 0.55 mmol) and 3-(pyridin-2-yl)propan-1-amine (0.07 g, 0.55 mmol) to give **36** (0.060 g, 29%) as an off-white solid after silica gel column purification. LCMS (ESI): m/z = 374.10 [M + H]⁺. HRMS (ESI⁺): m/z calcd for C₁₉H₁₈F₂N₃O₃⁺ (M + H)⁺, 374.1316; found, 374.1309. ¹H NMR (400 MHz, chloroform-*d*): δ 8.55 (d, *J* = 5.2 Hz, 1H), 8.12–8.02 (m, 2H), 7.82 (s, 1H), 7.66 (br s, 1H), 7.62–7.56 (m, 1H), 7.18 (d, *J* = 7.60 Hz, 1H), 7.13–7.10 (m, 1H), 4.07 (s, 3H), 3.51 (q, *J* = 6.50 Hz, 2H), 2.91 (t, *J* = 7.40 Hz, 2H), 2.14–2.05 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 161.02, 160.80, 155.04 (dd, *J* = 246 and 6.7 Hz), 150.27, 149.28, 147.84, 137.74 (m), 136.49, 129.44, 122.92, 121.41 (m), 121.19, 112.38 (m), 61.73, 38.94, 35.57, 29.13. Purity: HPLC *t*_R = 5.81 min, HPLC purity > 99.0%.

5-(3-Fluoro-4-methoxyphenyl)-N-(3-(pyridin-2-yl)propyl)-oxazole-4-carboxamide (37, OCM-37).—The title compound was synthesized from 5-(3-fluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and 3-(pyridin-2-yl)propan-1-amine (0.37 g, 1.00 mmol) to give **37** (0.130 g, 44%), as an off-white solid. LCMS (ESI): m/z = 356.10 [M + H]⁺. HRMS (ESI⁺): m/z calcd for C₁₉H₁₉FN₃O₃⁺ (M + H)⁺, 356.1410; found, 356.1404. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.54 (s, 1H), 8.52–8.46 (m, 2H), 8.35 (dd, *J* = 2.00, 13.60 Hz, 1H), 7.96 (d, *J* = 8.40 Hz, 1H), 7.68 (dt, *J* = 1.60, 7.60 Hz, 1H), 7.32–7.26 (m, 2H), 7.20–7.17 (m, 1H), 3.90 (s, 3H), 3.34–3.29 (m, 2H), 2.78–2.74 (m, 2H), 1.92 (quin, *J* = 7.40 Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 161.09, 160.65, 150.78 (d, *J* = 241.3 Hz), 149.80, 149.74, 148.92, 148.30 (d, *J* = 10.6 Hz), 136.38, 128.31, 124.44 (d, *J* = 3 Hz), 122.67, 121.15, 119.68 (d, *J* = 8.1 Hz), 115.49 (d, *J* = 21.5 Hz), 113.63, 56.10, 38.48, 35.03, 29.01. Purity: HPLC *t*_R = 5.51 min, HPLC purity > 99.2%.

5-(3-Fluoro-4-(trifluoromethyl)phenyl)-N-(3-(pyridin-3-yl)-propyl)oxazole-4-carboxamide (39, OCM-39).—The title compound was synthesized from 5-(3-fluoro-4-(trifluoromethyl)phenyl)oxazole-4-carboxylic acid (0.2 g, 0.72 mmol) and 3-(pyridin-3-yl)propan-1-amine (0.12 g, 0.87 mmol) to give **39** (0.045 g, 15%) as an off-white solid. LCMS (ESI): $m/z = 394.10$ $[M + H]^+$. HRMS (ESI⁺): m/z calcd for $C_{19}H_{16}F_4N_3O_2^+$ ($M + H$)⁺, 394.1179; found, 394.1172. ¹H NMR (400 MHz, chloroform-*d*): δ 8.60 (br s, 1H), 8.39 (d, $J = 11.6$ Hz, 1H), 8.26 (d, $J = 8$ Hz, 1H), 7.92 (s, 1H), 7.70 (t, $J = 8.00$ Hz, 1H), 7.57 (d, $J = 8.00$ Hz, 1H), 7.42 (m, 1H), 3.52 (q, $J = 6.8$ Hz, 2H), 2.76 (t, $J = 7.8$ Hz, 2H), 2.00 (quin, $J = 7.40$ Hz, 2H) (*1H merged in the solvent peak*). ¹³C NMR not assigned. Purity: HPLC $t_R = 6.34$ min, HPLC purity > 97.7%.

N-(3-(Pyridin-3-yl)propyl)-5-(3,4,5-trifluorophenyl)oxazole-4-carboxamide (40, OCM-40).—The title compound was synthesized from 5-(3,4,5-trifluorophenyl)oxazole-4-carboxylic acid (0.2 g, 0.82 mmol) and 3-(pyridin-3-yl)propan-1-amine (0.135 g, 0.98 mmol) to give **40** (0.070 g, 23%) as an off-white solid. LCMS (ESI): $m/z = 362.65$ $[M + H]^+$. HRMS (ESI⁺): m/z calcd for $C_{18}H_{15}F_3N_3O_2^+$ ($M + H$)⁺, 362.1116; found, 362.1116. ¹H NMR (400 MHz, chloroform-*d*): δ 8.50 (s, 1H), 8.47 (d, $J = 4.40$ Hz, 1H), 8.25–8.17 (m, 2H), 7.87 (s, 1H), 7.56 (d, $J = 8.00$ Hz, 1H), 7.40 (m, 1H), 7.26–7.23 (m, 1H), 3.52 (q, $J = 6.90$ Hz, 2H), 2.76–2.73 (m, 2H), 2.00 (quin, $J = 7.40$ Hz, 2H). ¹³C NMR not assigned. Purity: HPLC $t_R = 6.01$ min, HPLC purity > 99.6%.

5-(3,4-Difluorophenyl)-N-(3-(pyridin-3-yl)propyl)oxazole-4-carboxamide (41, OCM-41).—The title compound was synthesized from 5-(3,4-difluorophenyl)oxazole-4-carboxylic acid (0.150 g, 0.66 mmol) and 3-(pyridin-3-yl)propan-1-amine (0.11 g, 0.80 mmol) to give **41** (0.10 g, 43.5%) as an off white solid. LCMS (ESI): $m/z = 344.05$ $[M + H]^+$. HRMS (ESI⁺): m/z calcd for $C_{18}H_{16}F_2N_3O_2^+$ ($M + H$)⁺, 344.1211; found, 344.1201. ¹H NMR (400 MHz, chloroform-*d*): δ 8.49–8.46 (m, 2H), 8.39–8.34 (m, 1H), 8.16–8.13 (m, 1H), 7.85 (s, 1H), 7.54 (d, $J = 8.00$ Hz, 1H), 7.38 (br s, 1H), 7.30–7.20 (m, 2H), 3.51 (q, $J = 6.70$ Hz, 2H), 2.74 (t, $J = 7.80$ Hz, 2H), 2.03–1.95 (m, 2H). ¹³C NMR not assigned. Purity: HPLC $t_R = 5.72$ min, HPLC purity > 97.3%.

5-(3,5-Difluoro-4-methoxyphenyl)-N-(3-(pyridin-3-yl)propyl)-oxazole-4-carboxamide (42, OCM-42).—The title compound was synthesized from 5-(3,5-difluoro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.78 mmol) and 3-(pyridin-3-yl)propan-1-amine (0.127 g, 0.93 mmol) to give **42** (0.1 g, 34.6%) as an off-white solid. LCMS (ESI): $m/z = 374.15$ $[M + H]^+$. HRMS (ESI⁺): m/z calcd for $C_{19}H_{18}F_2N_3O_3^+$ ($M + H$)⁺, 374.1316; found, 374.1321. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.61 (s, 1H), 8.60–8.56 (m, 1H), 8.50–8.35 (m, 2H), 8.18–8.06 (m, 2H), 7.64 (d, $J = 7.60$ Hz, 1H), 7.29 (m, 1H), 4.00 (s, 3H), 3.31–3.26 (m, 2H), 2.62 (t, $J = 7.60$ Hz, 2H), 1.90–1.80 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 160.42, 154.39 (dd, $J = 244$ and 6.7 Hz), 150.37, 149.57, 148.50, 147.10, 136.96 (m), 136.79 (d, $J = 13.9$ Hz), 135.75, 129.66, 123.45, 121.64 (m), 112.09 (m), 61.75, 38.29, 30.35, 29.60. Purity: HPLC $t_R = 5.90$ min, HPLC purity > 99.4%.

5-(3-Fluoro-4-methoxyphenyl)-N-(3-(pyridin-3-yl)propyl)-oxazole-4-carboxamide (44, OCM-44).—The title compound was synthesized from 5-(3-fluoro-4-

methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.84 mmol) and 3-(pyridin-3-yl)propan-1-amine (0.137 g, 1.00 mmol) to give **44** (0.145 g, 48%) as an off-white solid. LCMS (ESI): $m/z = 356.10$ $[M + H]^+$. HRMS (ESI⁺): m/z calcd for $C_{19}H_{19}FN_3O_3^+$ ($M + H$)⁺, 356.1410; found, 356.1402. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.55 (s, 1H), 8.49 (t, $J = 5.60$ Hz, 1H), 8.45 (d, $J = 2.00$ Hz, 1H), 8.39 (dd, $J = 1.40, 4.80$ Hz, 1H), 8.35 (dd, $J = 2.20, 13.20$ Hz, 1H), 7.97 (d, $J = 8.40$ Hz, 1H), 7.66–7.64 (m, 1H), 7.32–7.28 (m, 2H), 3.91 (s, 3H), 3.34–3.26 (m, 2H), 2.63 (t, $J = 7.80$ Hz, 2H), 1.84 (quin, $J = 7.34$ Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 161.09, 160.65, 150.78 (d, $J = 241.3$ Hz), 149.80, 149.74, 148.92, 148.30 (d, $J = 10.6$ Hz), 136.38, 128.31, 124.44 (d, $J = 3$ Hz), 122.67, 121.15, 119.68 (d, $J = 8.1$ Hz), 115.49 (d, $J = 21.5$ Hz), 113.64, 56.08, 38.20, 30.38, 29.58. Purity: HPLC $t_R = 5.55$ min, HPLC purity > 95.4%.

5-(3-Chloro-4-methoxyphenyl)-N-(3-(pyrazin-2-yl)propyl)-oxazole-4-carboxamide (45, OCM-45).—

The title compound was synthesized from 5-(3-chloro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.12 g, 0.5 mmol) and 3-(pyrazin-2-yl)propan-1-amine (0.084 g, 0.61 mmol) to give **45** (0.060 g, 31%) as an off-white solid. LCMS (ESI): $m/z = 373.15$ $[M + H]^+$. HRMS (ESI⁺): m/z calcd for $C_{18}H_{18}ClN_4O_3^+$ ($M + H$)⁺, 373.1067; found, 373.1064. ¹H NMR (400 MHz, chloroform-*d*): δ 8.51–8.50 (m, 2H), 8.41 (d, $J = 2.00$ Hz, 1H), 8.38 (dd, $J = 2.0, 8.80$ Hz, 1H), 8.33 (d, $J = 2.00$ Hz, 1H), 7.81 (s, 1H), 7.48 (br s, 1H), 7.01 (d, $J = 8.80$ Hz, 1H), 3.96 (s, 3H), 3.54 (q, $J = 6.80$ Hz, 2H), 2.94 (t, $J = 7.60$ Hz, 2H), 2.16–2.09 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 161.12, 156.71, 156.16, 151.45, 147.52, 144.60, 144.01, 142.36, 129.82, 128.44, 128.11, 122.45, 120.36, 111.53, 56.21, 38.66, 32.65, 28.84. Purity: HPLC $t_R = 7.84$ min, HPLC purity > 99.8%.

5-(3-Chloro-4-methoxyphenyl)-N-(3-(pyridin-2-yl)propyl)-oxazole-4-carboxamide (46, OCM-46).—

The title compound was synthesized from 5-(3-chloro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.79 mmol) and 3-(pyridin-2-yl)propan-1-amine (0.13 g, 0.95 mmol) to give **46** (0.090 g, 30%) as an off-white solid. LCMS (ESI): $m/z = 372.25$ $[M + H]^+$. HRMS (ESI⁺): m/z calcd for $C_{19}H_{19}ClN_3O_3^+$ ($M + H$)⁺, 372.1115; found, 372.1116. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.53–8.45 (m, 4H), 8.10 (dd, $J = 2.00, 8.60$ Hz, 1H), 7.66 (dt, $J = 2.00, 7.60$ Hz, 1H), 7.28–7.25 (m, 2H), 7.18–7.15 (m, 1H), 3.91 (s, 3H), 3.33–3.28 (m, 2H), 2.74 (t, $J = 7.40$ Hz, 2H), 1.91 (quin, $J = 7.30$ Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 161.10, 160.63, 155.47, 149.83, 149.65, 148.94, 136.40, 129.31, 128.33, 127.91, 122.69, 121.17, 120.93, 120.30, 112.64, 56.31, 38.48, 35.04, 29.05. Purity: HPLC $t_R = 5.82$ min, HPLC purity > 98.8%.

5-(3-Chloro-4-methoxyphenyl)-N-(3-(pyridin-3-yl)propyl)-oxazole-4-carboxamide (47, OCM-47).—

The title compound was synthesized from 5-(3-chloro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.79 mmol) and 3-(pyridin-3-yl)propan-1-amine (0.13 g, 0.95 mmol) to give **47** (0.12 g, 40.5%) as an off-white solid. LCMS (ESI): $m/z = 372.20$ $[M + H]^+$. HRMS (ESI⁺): m/z calcd for $C_{19}H_{19}ClN_3O_3^+$ ($M + H$)⁺, 372.1115; found, 372.1107. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.55 (s, 1H), 8.53 (d, $J = 2.00$ Hz, 1H), 8.50–8.45 (m, 2H), 8.39 (d, $J = 3.60$ Hz, 1H), 8.11 (dd, $J = 2.00, 8.80$ Hz, 1H), 7.65 (d, $J = 7.60$ Hz, 1H), 7.31–7.28 (m, 2H), 3.92 (s, 3H), 3.26–3.27 (m, 2H), 2.63 (t, $J = 7.80$ Hz, 2H), 1.84 (quin, $J = 7.30$ Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 160.67, 155.47, 149.80,

149.67, 149.58, 147.09, 137.03, 135.75, 129.29, 128.30, 127.90, 123.36, 120.93, 120.28, 112.63, 56.30, 38.22, 30.44, 29.61. Purity: HPLC t_R = 5.88 min, HPLC purity > 98.8%.

5-(3-Chloro-4-methoxyphenyl)-N-(2-(5-fluoro-1H-indol-1-yl)-ethyl)oxazole-4-carboxamide (48, OCM-48).—The title compound was synthesized from 5-(3-chloro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.2 g, 0.79 mmol) and 2-(5-fluoro-1H-indol-1-yl)ethan-1-amine (0.17 g, 0.95 mmol), and the crude residue obtained was purified by the Grace reverse phase system (40 g C-18 cartridge, gradient 0–70% ACN in H₂O) and lyophilized to give **48** (0.09 g, 28%) as an off-white solid. LCMS (ESI): m/z = 414.10 [M + H]⁺. HRMS (ESI⁺): m/z calcd for C₂₁H₁₈ClFN₃O₃⁺ (M + H)⁺, 414.1021; found, 414.1009. ¹H NMR (400 MHz, chloroform-*d*): δ 8.37–8.32 (m, 2H), 7.75 (s, 1H), 7.34–7.28 (m, 2H), 7.15 (d, J = 3.42 Hz, 1H), 7.04 (d, J = 9.29 Hz, 1H), 6.97–6.91 (m, 1H), 6.47 (d, J = 2.93 Hz, 1H), 4.37 (t, J = 6.11 Hz, 2H), 3.98 (s, 3H), 3.81 (q, J = 6.20 Hz, 2H). ¹³C NMR (100 MHz, chloroform-*d*): δ 161.5, 157.9 (d, J = 234 Hz), 156.3, 151.8, 147.6 (d, J = 3.82 Hz), 132.6, 129.9, 129.5, 128.9 (d, J = 10.60 Hz), 128.4, 127.7, 122.6, 120.2, 111.6, 110.1 (d, J = 26 Hz), 109.9 (d, J = 26 Hz), 105.8 (d, J = 9 Hz), 101.7, 56.3, 46.0, 39.6. Purity: HPLC t_R = 8.99 min, HPLC purity > 98.8%.

5-(3-Chloro-4-methoxyphenyl)-N-(3-(5-fluoropyridin-3-yl)-propyl)oxazole-4-carboxamide (49, OCM-49).—The title compound was synthesized from 5-(3-chloro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.1 g, 0.39 mmol) and 3-(5-fluoropyridin-3-yl)propan-1-amine (0.07 g, 0.47 mmol), and the crude residue obtained was purified by column chromatography (230–400 mesh silica gel gradient 1–50% EtOAc in *n*-hexane) to give **49** (0.077 g, 50%) as an off-white solid. LCMS (ESI): m/z = 390.05 [M + H]⁺. HRMS (ESI⁺): m/z calcd for C₁₉H₁₈ClFN₃O₃⁺ (M + H)⁺, 390.1021; found, 390.1026. ¹H NMR (400 MHz, chloroform-*d*): δ 8.37 (dd, J = 2.20, 8.56 Hz, 1H), 8.35–8.28 (m, 3H), 7.80 (s, 1H), 7.37–7.27 (m, 2H), 7.01 (d, J = 8.80 Hz, 1H), 3.96 (s, 3H), 3.51 (q, J = 6.68 Hz, 2H), 2.75 (t, J = 7.83 Hz, 2H), 2.04–1.96 (m, 2H). ¹³C NMR (100 MHz, chloroform-*d*): δ 161.1, 159.46 (d, J = 255 Hz), 156.2, 151.5, 147.5 (d, J = 4 Hz), 145.6 (d, J = 3 Hz), 138.5 (d, J = 4 Hz), 135.9 (d, J = 23 Hz), 129.8, 128.4, 128.0, 122.5 (d, J = 17 Hz), 122.4, 120.3, 111.5, 56.2, 38.6, 30.8, 29.9. Purity: HPLC t_R = 8.61 min, HPLC purity > 99.3%.

5-(3-Chloro-4-methoxyphenyl)-N-(3-(6-fluoropyridin-3-yl)-propyl)oxazole-4-carboxamide (50, OCM-50).—The title compound was synthesized from 5-(3-chloro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.1 g, 0.39 mmol) and 3-(6-fluoropyridin-3-yl)propan-1-amine (0.07 g, 0.47 mmol), and the crude residue obtained was purified by column chromatography (gradient 0–70% EtOAc in *n*-hexane) to give **50** (0.05 g, 33%) as an off-white solid. LCMS (ESI): m/z = 390.05 [M + H]⁺. HRMS (ESI⁺): m/z calcd for C₁₉H₁₈ClFN₃O₃⁺ (M + H)⁺, 390.1021; found, 390.1015. ¹H NMR (400 MHz, chloroform-*d*): δ 8.38 (dd, J = 1.96, 8.80 Hz, 1H), 8.35 (d, J = 1.96 Hz, 1H), 8.07 (s, 1H), 7.82 (s, 1H), 7.67–7.62 (m, 1H), 7.34 (br s, 1H), 7.02 (d, J = 8.80 Hz, 1H), 6.87 (dd, J = 2.45, 8.31 Hz, 1H), 3.97 (s, 3H), 3.51 (q, J = 6.85 Hz, 2H), 2.73 (t, J = 7.83 Hz, 2H), 2.01–1.93 (m, 2H). ¹³C NMR (100 MHz, chloroform-*d*): δ 162.5 (d, J = 237.6 Hz), 161.2, 156.3, 151.6, 147.6 (d, J = 4.24 Hz), 147.0 (d, J = 12.29 Hz), 141.0 (d, J = 7.21 Hz), 134.2 (d, J = 4.24 Hz),

129.9, 128.5, 128.0, 122.5, 120.3, 111.6, 109.3 (d, $J = 37.1$ Hz), 56.2 (d, $J = 3.39$ Hz), 38.6, 31.1, 29.5. Purity: HPLC $t_R = 8.91$ min, HPLC purity > 99.8%.

N-(3-(5-Fluoropyridin-3-yl)propyl)-5-(3-iodo-4-methoxyphenyl)-oxazole-4-carboxamide (51, OCM-51).—The title compound was synthesized from 5-(3-chloro-4-methoxyphenyl)oxazole-4-carboxylic acid (0.10 g, 0.29 mmol), and the crude residue obtained was purified by column chromatography (gradient 0–70% EtOAc in *n*-hexane) to give **51** (0.041 g, 29%) as an off-white solid. LCMS (ESI): $m/z = 482.00$ [$M + H$]⁺. HRMS (ESI⁺): m/z calcd for C₁₉H₁₈FIN₃O₃⁺ ($M + H$)⁺, 482.0377; found, 482.0370. ¹H NMR (400 MHz, chloroform-*d*): δ 8.64 (d, $J = 1.96$ Hz, 1H), 8.50 (dd, $J = 1.96, 8.80$ Hz, 1H), 8.33–8.29 (m, 2H), 7.80 (s, 1H), 7.33 (br s, 1H), 7.28 (br s, 1H), 6.90 (d, $J = 8.80$ Hz, 1H), 3.94 (s, 3H), 3.51 (q, $J = 6.85$ Hz, 2H), 2.75 (t, $J = 7.83$ Hz, 2H), 2.02–1.93 (m, 2H). ¹³C NMR (100 MHz, chloroform-*d*): δ 160.7, 159.5 (d, $J = 255.0$ Hz), 159.3, 150.8, 147.1 (d, $J = 4.66$ Hz), 145.2, 138.4, 138.1 (d, $J = 3.39$ Hz), 135.8 (d, $J = 23.2$ Hz), 129.9, 127.4, 122.5 (d, $J = 17.70$ Hz), 120.9, 109.8, 85.1, 56.0, 38.2, 30.4, 29.5. Purity: HPLC $t_R = 8.94$ min, HPLC purity > 99.8%.

Radioligand Saturation Binding Assay.

[³H]**1** (81 Ci/mmol (3.0 TBq/mmol) molar activity, 99.9% radiochemical purity) was provided by MedChem Imaging, Inc. For additional details, see Supporting Information Materials and Methods.

HEK-293T P301S hTau cellular Assay.

HEK-293T P301S hTau cellular assay was performed as described in the Supporting Information Materials and Methods.

In Vitro Autoradiography.

For autoradiography experiments, fresh-frozen postmortem human brain tissue blocks were acquired from Folio-Conversant and the University of California at San Francisco NDBB. In vitro autoradiography studies were performed as described in the Supporting Information Materials and Methods. Phosphorimaging of tissue sections was performed using Amersham Typhoon (GE Healthcare, MA).

Docking Studies.

Molecular docking simulations of **44** and **51** were performed using the X-ray cocrystal structure of GSK-3 β complexed with **1** (PDB code: 5K5N) and GSK-3 β complexed with BRD0209 (PDB code: 5KPK) using the FITTED 3.5 program (FORECASTER platform).^{49–51} Docking structures and figures were prepared using Pymol.

Kinase Assays.

ADPGlo binding studies were performed as previously described.²⁸ In vitro radiometric kinase assay including K_i determination and kinome selectivity profiles for selected inhibitors was obtained through [γ -³³P]ATP-based enzymatic assays (Reaction Biology Corporation, Malvern, PA).³⁰ For additional details, see the Supporting Information

Materials and Methods. Detailed results for kinome selectivity can also be found in Tables S5–S7. Chemical proteomic (KiNativ) profiling using brain lysate was performed by ActivX Bioscience (La Jolla, CA). Detailed results for the kinativ profiling can be found in Table S8.

CNS Target-Binding Assays.

Inhibitor **51** was tested against a panel of 80 binding assays encompassing common targets found in the CNS (LeadProfilingScreen2 Panel, Eurofins Scientific). Detailed results for the CNS target-binding assay profiling are found in Table S9. The IC₅₀s for the five main off-target of **51** (5-HT_{2B}, BZD, Cl[−] channel (GABA-gated), KOP, NK1) were determined using radio-ligand-binding assays (Eurofins Scientific). K_i values were calculated using the Cheng–Prusoff equation.

MST Assay.

The MST assay was performed at the Center for Macromolecule Interaction, Harvard University. For additional details, see the Supporting Information Materials and Methods.

hERG Assay.

hERG assays were performed at Reaction Biology Corporation (Malvern, PA) as previously described.

WNT Reporter Assay.

The WNT reporter assay was performed as described in the Supporting Information Materials and Methods and as previously described.⁴¹

NPC Proliferation/Viability Assay.

The NPC proliferation/viability assay was performed as described in the Supporting Information Materials and Methods.

CRMP2 and pTau Assays.

The CRMP2 and pTau assays were performed as described in Supporting Information Materials and Methods and as previously described.^{38,40}

Western Blot Analysis.

The Western blot analysis was performed as described in the Supporting Information Materials and Methods.

Radiochemical Syntheses (44).

Detailed synthetic procedures for all radiotracers are provided in Scheme S4 and Supporting Information Materials and Methods. Briefly, for the radiosynthesis of [¹¹C]**44**, [¹¹C]CH₃OTf (obtained from ~1000 mCi of [¹¹C]CH₃I) was bubbled through a reaction solution containing 1.0 mg of the corresponding desmethyl precursor, 5.0 μL of 1.0 M methanolic TBAOH, and 100 μL of DMF at room temperature for 3 min at 15 mL min^{−1} with Ar carrier gas. The reaction was quenched with 1000 μL 50/50 MeCN/10mM NH₄OAc (pH: 7.3) and

loaded onto HPLC (50/50 MeCN/20 mM NH₄OAc (pH: 7.3) (v/v) isocratic; flow rate: 5.0 mL/min; semipreparative column: Phenomenex Luna C18, 10 μ m, 10 $\times$ 250 mm, 5 mL/min). The peak between 7.5 and 8.1 min corresponding to [¹¹C]**44** (Figure S16) was collected and diluted with 50 mL H₂O, and the solution was transferred through preconditioned C18 1cc Vac Sep Pak. The radiotracer was eluted with 500 μ L EtOH. The C18 cartridge was then washed with 4.5 mL buffered saline. Then, the product was passed through a sterile filter and into the product vial. Pure [¹¹C]**44** was obtained in ~1% nondecay-corrected radiochemical yields at end-of-synthesis based upon [¹¹C]CH₃I, radiochemical purity > 99% determined by radio-HPLC, A_m = 264 GBq/ μ mol (NHP experiment), and A_m = 426 GBq/ μ mol (rodent experiment). Quality control mobile phase: 50/50 MeCN/10mM NH₄OAc (pH: 7.3) (v/v) isocratic; flow rate: 1.0 mL/min; QC column: Phenomenex Luna C18, 5 μ m, 4.6 $\times$ 150 mm (Figures S17 and S18).

Rodents and Nonhuman Primate PET Imaging Studies (1, 28, 37, 44).

All imaging studies were performed in accordance with the standards set by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. Rat imaging was done using Sprague Dawley (body weight $\approx$ 297–339 g). NHP imaging was done using a mature female rhesus monkey (*Macaca mulatta*) (body weight $\approx$ 8.8 kg with negligible variation throughout the duration of the study, 15 years of age). Imaging procedure and analysis is described in the Supporting Information Materials and Methods.

Neuropharmacokinetic Analysis.

NeuroPK was performed at Sai Life Sciences as described in the Supporting Information Materials and Methods.

Statistical Analysis.

Data were expressed as mean $\pm$ SD. Statistical analysis was performed using GraphPad Prism 7.0c. *p* values less than 0.05 were considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank Dr. Kelly L. Arnett and Harvard University's Center for Macromolecular Interaction for advice regarding MST and Dr. Poguang Wang for HRMS analyses. Funding to S.J.H. provided by the Tau Consortium and MGH Research Scholars Program. We thank Smita Jacob for graphic design. N.V. thanks National Institute on Ageing of the NIH (R01AG054473), the Azrieli Foundation and the Canada Research Chairs Program for support.

ABBREVIATIONS

AD	Alzheimer's disease
ADME	absorption; distribution, metabolism and elimination
ADP	adenosine 5'-diphosphate

AKT	protein kinase B
A_m	molar activity
APP	amyloid precursor protein
Ar	argon
ATP	adenosine 5'-triphosphate
AUC_{last}	area under time–concentration curve to last measurable concentration
BBB	blood–brain barrier
B_{max}	binding site density
BZD	benzodiazepine
CB	cerebellum
CC	corpus callosum
Cl	chloro
C_{max}	maximum serum concentration
CNS	central nervous system
CPu	caudate putamen
CRMP	collapsing response mediator protein
Ctx	cortex
DMF	dimethylformamide
ESI	electrospray ionization
EtOH	ethanol
Fu_p	unbound brain fraction
GABA	γ-aminobutyric acid
GBq	gigabecquerel
GM	grey matter
GSK-3	glycogen synthase kinase-3
hERG	human ether-a-go-go related gene
HP	hippocampus
HT	hydroxytryptamine
IC₅₀	half-maximum inhibitory concentration

K_D	dissociation constant
K_i	inhibition constant
K_M	Michaelis constant
KOP	kappa opioid receptor
$K_{p,u}$	unbound partition coefficient
LCMS	liquid chromatography–mass spectrometry
mCi	millicuries
MeCN	acetonitrile
MPO	multi-parameter optimization
MST	microscale thermophoresis
NH₄OAc	ammonium acetate
NHP	nonhuman primate
NIH	National Institute of Health
NK1	neurokinin 1
NMR	nuclear magnetic resonance
NPC	neural progenitor cell
PDB	Protein Data Bank
PET	positron emission tomography
PF-367	PF-04802367
pGS	phosphorylated glycogen synthase
PO	oral gavage
pTau	hyperphosphorylated tau protein
SAR	structure–activity relationship
SD	standard deviation
SUV	standard uptake value
TAC	time activity curves
TBAOH	tetrabutylammonium hydroxide
Tg	transgenic
T_{max}	time that maximum serum concentration is observed

UCUCA	University Committee on Use and Care of Animals
WM	white matter

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Figure 1.

[³H]PF-04802367 (**1**) displays high specific and selective binding to GSK-3 α/β in cells and mammalian brains *in vitro*. (A) Chemical structure of [³H]**1**. (B) Quantification for the *in vitro* autoradiography of [³H]**1** in the normal rat brain (20 μ m sections, $n = 3$) under baseline, homologous blocking (nonradioactive **1**, 10 μ M), and heterologous (LY2090314, 10 μ M or CHIR99021, 10 μ M) conditions. (C) *In vitro* autoradiography images of [³H]**1** normal rat brain (coronal sections) under baseline, homologous blocking (nonradioactive **1**, 10 μ M), and heterologous (LY2090314, 10 μ M or CHIR99021, 10 μ M) conditions. (D) Quantification for the *in vitro* autoradiography of [³H]**1** in the normal marmoset brain (20 μ m sections, $n = 3$) under baseline, homologous blocking (nonradioactive **1**, 10 μ M), and heterologous (LY2090314, 10 μ M or CHIR99021, 10 μ M) conditions. (E) Quantification for the *in vitro* autoradiography of [³H]**1** in the normal human brain (20 μ m sections, frontal cortex, $n = 6$) under baseline, homologous blocking (nonradioactive **1**, 10 μ M), and

heterologous (LY2090314, 10 μ M or CHIR99021, 10 μ M) conditions. (F) In vitro autoradiography images of [3 H]**1** normal human brain under baseline, homologous blocking (nonradioactive **1**, 10 μ M), and heterologous (LY2090314, 10 μ M or CHIR99021, 10 μ M) conditions. (G) Saturation [3 H]**1** binding curves, Scatchart plot, and $B_{\max}$ determination using the homogenate mouse brain tissue. (H,I) Cell binding assays with [3 H]**1** in HEK-293T P301S hTau cells, overexpressing GSK-3 α/β and GSK-3 α/β knockdown (shRNA). [3 H]**1** binding under 2h baseline and following timecourse chase experiments. (J) Western blot analysis of control, GSK-3 α/β -overexpressing, and GSK-3 α/β shRNA cells used for [3 H]**1**-binding studies. Data show mean values $\pm$ standard deviation (SD). Statistical significance was calculated one-way ANOVA followed by Dunnett's test (B,D,E,H).

Figure 2.

Evolution and in vitro pharmacological characterization of a highly potent and selective oxazole-4-carboxamide-based GSK-3 inhibitor series. (A) Generalized strategy to synthesize arrays of carboxamide-based GSK-3 inhibitors, as well as structures and properties of selected GSK-3 inhibitors (for details on chemical synthesis, see Schemes S1–S4). IC₅₀ values determined using an ADP-Glo kinase assay ($n = 2$). (B) Heat map representing key properties [IC₅₀, central nervous system multiparameter optimization (CNS MPO), and Fu_p] of oxazole-4-carboxamide-based GSK-3 inhibitors including a comparison with previously described GSK-3 PET radiotracers (for structures and detailed parameter values, see Tables S1–S4). IC₅₀ values determined using an ADP-GloTM kinase assay ($n = 2$). (C,D) The dose–response curves and binding affinities of the representative inhibitor 28 versus GSK-3α, GSK-3β ($n = 3$), and main kinase off targets DYRK1A, DYRK1B, and CLK4 ($n = 1$) demonstrate high selectivity for GSK-3α/β (radiometric filtration binding assay, [ATP] = K_m ATP, data show mean values ± SD). (E) Hinge/α2 region sequence alignment of GSK-3β and the main kinase secondary target DYRK1A. (F) Comprehensive kinase selectivity profile of representative inhibitor 44 and 28 against 371 kinases (kinases are ordered alphabetically, and the activity is relative to control at 1.0 μM inhibitor concentration, $n = 2$, [γ -³³P]ATP-based enzymatic assay performed by Reaction Biology, for detailed data see Table S5). (G) Overlay of the hinge/α2 region of GSK-3β (gray, PDB ID code 5K5N) and DYRK1A (yellow, PDB ID code 3ANR). (H) Predicted detailed interaction of **44** (yellow sticks) with GSK-3β (gray cartoon, PDB ID code 5K5N). The π -cation interaction is depicted as a black dash line. (I) Alternate view of the binding mode of **44** (yellow, space fill model) with GSK-3β (surface rendering, PDB ID code 5K5N). CNS MPO; Fu_p, in silico calculated unbound fraction in plasma.

Figure 3.

Oxazole-4-carboxamide-based probes engage both active npS9 and catalytically inactive pS9 GSK-3 β . (A) Structural basis for the recognition of inhibitors by pS9-GSK-3 β (**44** is depicted as a representative inhibitor, yellow, space fill model). The predicted binding mode illustrates the distal positioning of **44** from the activation loop and pS9 auto-inhibitory N-terminal peptide (blue surface) and the negligible ATP-binding site rearrangement upon binding of the pS9 auto-inhibitory N-terminal peptide of GSK-3 β (gray surface, PDB ID code 4NM3). (B) Timecourse analysis showing GSK-3 β activity in the presence of low ATP content and GSP2 (ADP-Glo-based kinase assay, $n = 3$). (C) Timecourse analysis showing GSK-3 β activity in the presence of high ATP content and high ATP content combined with AKT (ADP-Glo-based kinase assay, $n = 3$). (D) Western blot analysis of enzymatic reaction contents (control, ATP treated and ATP + AKT treated). (E) Binding affinities of **1** and **44** measured by MST. (F,G) MST dose-response curves for the binding interaction of between **1** with active npS9-GSK-3 β and pS9-GSK-3 β ($n = 2$). (H,I) MST dose-response curves for the binding interaction of **44** with active npS9-GSK-3 β and pS9-GSK-3 β ($n = 2$). Data show mean values $\pm$ SD. Statistical significance was calculated with paired t -test (B,C).

Figure 4.

Structure-guided design and characterization of the GSK-3 β isozyme-selective inhibitors **51**. (A) Structures and properties of selected GSK-3 inhibitors leading to **51** (for details on chemical synthesis, see Schemes S1–S4). IC₅₀ values determined using an ADP-Glo kinase assay ($n = 2$). (B) Dose–response curves of **51** versus GSK-3 α and GSK-3 β ($n = 3$) (compared with **28**, radiometric filtration binding assay, [ATP] = K_m ATP, data show mean values $\pm$ SD). (C) Predicted binding mode of **51** (green sticks) with GSK-3 β (gray cartoon, PDB ID code 5K5N and 5KPK). Highlight on the interaction network associated with Asp133. Hydrogen bonds are depicted as a yellow dash lines. (D) Binding affinity measurements of inhibitors versus GSK-3 α and GSK-3 β ($n = 3$). (E–G) Alternate views of the predicted contact between **51** and the selectivity pocket of GSK-3 β . (H) Kinativ data from mice brain lysates showing the selective engagement of the representative inhibitor **49** (1.0 μ M) with GSK-3 ($n = 2$, for detailed data, see Table S5). (I) Comprehensive kinase

selectivity profile of representative inhibitor **51** against 371 kinases (kinases are ordered alphabetically, and the activity is relative to control at 1.0 μM inhibitor concentration, $n = 2$, [γ - ^{33}P]ATP-based enzymatic assay performed by Reaction Biology, for detailed data see Table S9). (J) Selectivity profile of **51** (10.0 μM) against a CNS target panel ($n = 2$, 80 targets). (K) Binding affinity of **51** against the five most inhibited CNS targets at a concentration 100000-fold over the K_i of **51** for GSK-3 β . (L) MST dose-response curve and binding affinity for the interaction between **51** with active npS9-GSK-3 β and pS9-GSK-3 β ($n = 2$). (M) MST dose-response curve and binding affinity for the interaction between **51** with active npS9-GSK-3 β and pS9-GSK-3 β ($n = 2$). Data show mean values $\pm$ SD.

Figure 5.

Selective oxazole-4-carboxamide GSK-3 inhibitors modulate both pTau and pCRMP2^{T514} and display favorable therapeutic index against Wnt/ β -catenin signaling in human neuronal cells. (A–D) Dose-dependent effect of selected compounds on decreasing phosphorylated tau in human neurons. 20 h treatment with **1**, **28**, **51**, but not **29** dose-dependently decreased mean pTau (PHF-1) intensity in neuronal cell bodies. Each bar is the mean values ± SEM of 14–21 fields/well. Each dose of each compound was tested in 1 well per plate. Data from 3 plates in total are shown in the figure. (E–H) High-content imaging assays using human neurons showed that representative inhibitors (**1**, **28**, **51**) reduced CRMP2 phosphorylation in a dose-dependent manner whereas **29** did not. CRMP2 is a direct substrate of GSK-3 β . The relative level of pCRMP2^{T514} intensity was reported as the percentage of the intensity for dimethylsulfoxide (DMSO)-treated (100%) ($n = 3$ biological replicates, $n = 18$ images for analysis. Data show mean values ± SEM). Relative levels of dendritic marker MAP2

were not affected by the compound treatments, indicating the health of neurons. (I) Representative images of **51** treatment on pCRMP2^{T514} intensity (green) versus MAP2 intensity (red) illustrate the dose-dependent pCRMP2^{T514} intensity reduction. (J–M) Pro-neurogenic potentials measured as NPC proliferation were detected for active oxazole-4-carboxamide GSK-3 inhibitors. Graphs show dose-dependent increases from PF-367, PF-618, and **51** but not **29**. NPC proliferation is expressed as the percentage of DMSO-treated (100%). ($n = 4$. Error bars display SEM. Unpaired t -test. *0.01 $p < 0.05$, **0.001 $p < 0.01$, ***0.0001 $p < 0.001$, **** $p < 0.0001$.) (N) Active oxazole-4-carboxamide GSK-3 inhibitors enhanced Wnt/ β -catenin signaling in human NPCs. Dose–response curves of representative inhibitors (**1**, **28**, **51**, and inactive **29**) with calculated EC₅₀s are shown. $n = 4$. Data show mean values $\pm$ SEM.

Figure 6.

Oxazole-4-carboxamide-based probes display robust brain exposure properties in vivo and differential target engagement in a mouse model of AD in vitro. (A) In vitro autoradiography images of [³H]**1** Tg2576 and C57BL/6 mice under baseline, homologous blocking (nonradioactive **1**, 10 μ M), and heterologous (LY2090314, 10 μ M or CHIR99021, 10 μ M) conditions. (B) Summed (5 to 30 min) PET images showing the distribution of [¹¹C]**28**, [¹¹C]**37**, and [¹¹C]**44** in comparison with [¹¹C]**1** in the rhesus monkey brain, following microdose injections. Image intensity displayed as 0–2 SUV scale. (C) Quantification for the in vitro autoradiography of [³H]**1** in Tg2576 (20 μ m sections, $n = 3$) and C57BL/6 (20 μ m sections, $n = 2$) mice brains under baseline, homologous blocking (nonradioactive **1**, 10 μ M), and heterologous (LY2090314, 10 μ M or CHIR99021, 10 μ M) conditions. (D) Representative rhesus monkey regional brain time–activity curves (TACs) after the intravenous injection of [¹¹C]**44**. (E) Representative rhesus monkey regional brain time–

activity curves (TACs) after intravenous injection of [^{11}C]**1**. (F) Mice exposure of **44** (C57BL/6 mice, 50 mg/kg, single dose PO, $n = 3$). Data show mean values $\pm$ SD.

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