Journal of Medicinal Chemistry



Article

# Synthesis, Structure Activity Relationships and Preclinical evaluation of Heteroaromatic Amides and 1,3,4-Oxadiazole Derivatives as 5-HT4 Receptor Partial Agonists

Ramakrishna Nirogi, Abdul Rasheed Mohammed, Anil K. Shinde, Shankar Reddy Gagginapally, Durga Malleshwari Kancharla, Vanaja Reddy Middekadi, Narsimha Bogaraju, Srinivasa Rao Ravella, Pooja Singh, Sumit Raosaheb Birangal, Ramkumar Subramanian, Raghava Choudary Palacharla, Vijay Benade, Nageswararao Muddana, and Pradeep Jayarajan

*J. Med. Chem.*, **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.8b00457 • Publication Date (Web): 15 May 2018



## Just Accepted



ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

# Synthesis, Structure Activity Relationships and Preclinical evaluation of Heteroaromatic Amides and 1,3,4-Oxadiazole Derivatives as 5-$HT_4$ Receptor Partial Agonists

*Ramakrishna Nirogi,* Abdul Rasheed Mohammed, Anil K. Shinde, Shankar Reddy Gagginapally, Durga Malleshwari Kancharla, Vanaja Reddy Middekadi, Narsimha Bogaraju, Srinivasa Rao Ravella, Pooja Singh, Sumit Raosaheb Birangal, Ramkumar Subramanian, Raghava Choudary Palacharla, Vijay Benade, Nageswararao Muddana, Pradeep Jayarajan.*

Suven Life Sciences Limited, Discovery Research, Serene Chambers, Road-5, Avenue-7, Banjara Hills, Hyderabad - 500 034, India

KEYWORDS. 5-$HT_4$ receptor, Structure Activity Relationships, Pharmacokinetic profile, Receptor occupancy, Cognitive deficits, Alzheimer's disease, GR-125487

ABSTRACT. Alzheimer's disease (AD) is a neurodegenerative disorder which has a higher prevalence and incidence in people older than 60 years. The need for improved AD therapies is unmet as the current therapies are symptomatic with modest efficacy. The 5-$HT_4$ receptor (5-$HT_4$R) partial agonists offer both symptomatic and disease modifying treatment as they shift amyloid precursor protein (APP) processing from amyloidogenic to non-amyloidogenic pathway

by activating α-secretase enzyme. In addition, they also offer symptomatic treatment by increasing neurotransmitter acetylcholine levels in the brain. Fascinated by dual mechanism of action, several chemical scaffolds having 5-$HT_4$R pharmacophore were designed and evaluated. Most of the synthesized compounds showed potent *in vitro* affinities and *in vivo* efficacies. Upon focused structure activity relationships, compound **4o** was identified as potent 5-$HT_4$R partial agonist with favorable ADME properties and good *in vivo* efficacy. GR-125487, a selective 5-$HT_4$R antagonist attenuated the activity of compound **4o** in cognition model NORT.

## INTRODUCTION

AD is a progressively debilitating neurodegenerative disorder, affecting mostly the aging population. Although the underlying causative factors are yet to be identified, symptoms include confusion, memory loss, and dementia, ultimately leading to death. The current pharmacotherapy provides only symptomatic relief through augmentation of cholinergic function with undesired side effects such as nausea, vomiting and bradycardia. Moreover, no disease modifying therapy is available to the patients till date. Thus, there is a need of new therapeutic targets for the treatment of this disorder and 5-$HT_4$R is one such receptor which has attracted lot of attention. The 5-$HT_4$R belongs to the superfamily of seven trans-membrane G-protein coupled receptors (GPCRs) and is coupled to G protein containing Gαs subunit.[1] The 5-$HT_4$R is reported to have potential role in many central nervous system (CNS) such as AD[2] and peripherally mediated disorders such as irritable bowel syndrome[2] and gastroparesis.[4] The 5-$HT_4$ receptors are highly expressed in brain regions like hippocampus, amygdala and cerebral cortex suggesting the involvement of receptor in cognitive processes.[5] The 5-$HT_4$R agonists modulate amyloid precursor protein (APP) derived peptides, amyloid beta (Aβ) and soluble amyloid precursor protein alpha (sAPPα).[6]

The sAPPα is non-amyloidogenic protein and it is reported to have potent neuroprotective role against neurotoxic effects of glutamate and β-amyloid.[6] Human 5-$HT_4$R isoforms are positively coupled to adenylyl cyclase production. Thus, activation of this G-protein coupled receptor activates adenylyl cyclase consequently increasing the production of cAMP. The increased cAMP in turn increases sAPPα release. The stimulatory effect of 5-$HT_4$R receptor on sAPPα release is mimicked by forskolin, a direct activator of adenylyl cyclase, and 8-bromo-cAMP (membrane permeant cAMP analogue) suggesting the involvement of adenylyl cyclase and cAMP in modulating sAPPα by 5-$HT_4$R.[7] The 5-$HT_4$R also increases the neuronal acetylcholine (ACh) levels in the brain.[8] Thus, 5-$HT_4$R partial agonists may be of use for both disease-modifying and symptomatic treatment of cognitive disorders. The 5-$HT_4$R owing to its dual mechanism of action to treat AD and other cognition related diseases became an attractive target for drug discovery. Several structurally diverse heteroaromatic derivatives[9-13] as 5-$HT_4$R agonists/partial agonists have been explored for both CNS and peripheral indications. The 5-$HT_4$R partial agonist PRX-03140 showed significant improvement of ADAS-cog score in Phase-2a study for AD.[14,15] Apart from this compound, several other compounds were in development such as BIMU-1,[16] RS-67333,[17] prucalopride[3] and PF-04995274[9] among others (Figure 1). In our continuous efforts in discovering novel small molecules to treat AD with diverse mechanism of actions,[18-20] we shifted our attention on 5-$HT_4$R agonists partly due to their dual mechanism of action and partly due to availability of proof of concept in the form of PRX-03140 phase-2 positive results. Our internally discovered clinical candidate SUVN-D4010, a 5-$HT_4$R partial agonist has completed Phase-1 clinical trials in healthy subjects[21] and also completed Phase-2 enabling long term safety studies. The structure of this compound has not been disclosed so far.

Most of the reported 5-$HT_4$R ligands, which have been explored for both CNS and peripheral indications belong to either benzamide or imidazole carboxamide scaffold. Previous work[19] from our group disclosed a series of 3-isopropylimidazo[1,5-*a*]pyridine carboxamide derivatives (**5a**, Series-1) as 5-$HT_4$R partial agonists which displayed cognition enhancing properties in animal models. However, the brain penetration of these compounds was poor ($C_{brain}/C_{plasma}$ = <0.3). As we are targeting the CNS receptor, low brain penetration in rat makes this scaffold, unsuitable for further development. Our goal was to identify a potent 5-$HT_4$R agonist with adequate brain penetration, acceptable ADME and preclinical efficacy as a possible drug candidate for the treatment of AD. The Series-1 compounds[19] were modified by keeping the basic pharmacophore and the isopropyl substituent unchanged (Figure 2) to obtain several diverse chemical scaffolds. The 5-$HT_4$R pharmacophore consists of an aromatic moiety, a coplanar carbonyl group or its bioisostere, and a voluminous substituent in the basic amino framework of the molecule.[22,23]

## RESULTS AND DISCUSSION

Successive modifications in the position of the nitrogen atom, position of isopropyl group, position of carboxamide group in heteroaromatic ring resulted in novel 1-isopropylimidazo[1,5-*a*]pyridine carboxamide derivatives (Series-2), 2-isopropylimidazo[1,2-*a*]pyridine carboxamide derivatives (Series-3), 2-isopropylpyrazolo[1,5-*a*]pyridine carboxamide derivatives (Series-4). The replacement of carboxamide group in each of the series with amide bioisostere resulted in 1,3,4-oxadiazole derivatives. The results of these modifications covering *in vitro* binding affinity, pharmacokinetic, hERG affinity, receptor occupancy and *in vivo* efficacy profiling constitute the subject matter of this manuscript.

**Chemistry**: The synthesis of heteroaromatic carboxylic acid $\mathbf{A_1}$ commenced from commercially available 2-bromopyridine **1** which was treated with *n*-BuLi followed by isobutyraldehyde to obtain compound **2**. The hydroxyl group in compound **2** was converted into a leaving group by mesylation and then was displaced with sodium azide to obtain azido compound **3**. The azide group in compound **3** was reduced with triphenylphosphine/$H_2O$ combination (Staudinger reaction)[24] which afforded amino compound **4**. The amino compound **4** was treated with ethyl chlorooxoacetate to obtain intermediate **5** which on cyclization with $POCl_3$ in 1,2-dichloroethane afforded imidazo[1,5-*a*]pyridine ethyl ester **6**. The ester **6** was hydrolyzed with NaOH and thus obtained sodium salt upon acidification yielded 1-isopropyl-imidazo[1,5-*a*]pyridine-3-carboxylic acid $\mathbf{A_1}$ (Scheme 1). The 2-isopropylimidazo[1,2-*a*]pyridine carboxylic acid $\mathbf{A_2}$ was synthesized starting from commercially available ethyl 2-aminonicotinate **7**, which on refluxing with 1-chloro-3-methyl-butan-2-one in ethanol yielded imidazo[1,2-*a*]pyridine ester derivative **8** which after hydrolysis afforded the corresponding acid $\mathbf{A_2}$ (Scheme 2). Synthesis of 2-isopropylpyrazolo[1,5-*a*]pyridine-7-carboxylic acid $\mathbf{A_3}$ started from reaction of 2-picoline **9** with isobutyryl chloride which afforded intermediate **10**. The intermediate **10** on cyclization with *O*-mesitylenesulfonylhydroxylamine, afforded 2-isopropylpyrazolo[1,5-*a*]pyridine **11** which on reaction with *n*-BuLi followed by carboxylation with carbon dioxide (dry ice) and acidification yielded acid $\mathbf{A_3}$ (Scheme 3).

The ethyl isonipecotate **12** was alkylated, either by reacting with alkyl halides or by reductive amination with aldehydes to obtain compounds **13a**, **13b** and **13c**. Compounds **13a**, **13b** and **13c** on ester hydrolysis with lithium hydroxide afforded lithium carboxylates **14a**, **14b** and **14c** respectively (Scheme 4). The acid hydrazides **15**, **16** and **18** were synthesized from

corresponding esters **6**, **8** and **17**. The ester **17** was obtained from acid $\mathbf{A_3}$ upon treatment with dry HCl in methanol (Scheme 5).

The acids $\mathbf{A_1}$, $\mathbf{A_2}$ or $\mathbf{A_3}$ were reacted with earlier reported[19] *N*-Boc substituted piperidine $\mathbf{B_1}$ or 3-aza-bicyclo[3.1.0]hexane amines $\mathbf{B_2}$ respectively in presence of TBTU or HATU to obtain the Boc protected carboxamide derivatives **19a**, **19b**, **21a**, **21b**, **23a**, **23b** which on Boc deprotection with dry HCl in isopropanol afforded compounds **20a**, **20b**, **22a**, **22b**, **24a**, **24b** respectively. These compounds upon *N*-alkylation, either by reacting with alkyl halides or reductive amination with aldehydes afforded 1-isopropylimidazo[1,5-*a*]pyridine carboxamides **2a-2g** or 2-isopropylimidazo[1,2-*a*]pyridine carboxamides **3a-3q** or 2-isopropylpyrazolo[1,5-*a*]pyridine-7-carboxamides **4a-4l** respectively (Scheme 6). The 1,3,4-oxadiazole derivatives **2h-2j**, **3r-3s**, **4m-4o** were synthesized by reacting hydrazides **15**, **16** or **18** with substituted lithium carboxylates **14a**, **14b** or **14c** in presence of $POCl_3$ (Scheme 7).

The isopropylimidazo[1,5-*a*]pyridine carboxamide derivatives **2a-2e** with different substitutions on piperidine ($\mathbf{B_1}$) nitrogen showed *in vitro* potency $EC_{50}$ <100 nM towards 5-$HT_4$R with partial agonist activity ($E_{max}$<100%, Table 1). Compounds with bulkier substitutions such as methylpiperidine (**2c**) and methylpyran (**2d**) on piperidine nitrogen showed increased potency ($EC_{50}$ <10 nM) as compared to simple substitution bearing compounds such as isopropyl (**2a**) and methoxypropyl (**2b**). Similarly, compounds with *N*-substituted 3-aza-bicyclo[3.1.0]hexane ring ($\mathbf{B_2}$) showed increased potency at 5-$HT_4$R as compared to simple piperidine ($\mathbf{B_1}$) counterparts. Compounds with methoxypropyl substitution (**2f**) and pyran substitution (**2g**) on nitrogen of 3-aza-bicyclo[3.1.0]hexane showed increased potency as compared to the piperidine counterparts **2b** and **2e** respectively.

The development and application of bioisosteres have been adopted as a fundamental tactical approach useful to address a number of aspects such as improving potency, enhancing selectivity, altering physical property, reducing or redirecting metabolism, eliminating or modifying toxicophores or acquiring novel intellectual property associated with drug candidates.[25] The 1,3,4-oxadiazoles derivatives[26] **2h-2j** as carboxamide bioisosteres were synthesized and evaluated. However, these compounds showed weak *in vitro* potency at 5-$HT_4$R. In brief, the carboxamide derivatives showed moderate *in vitro* potency and 1,3,4-oxadiazole derivatives showed weak potency towards 5-$HT_4$R.[27] Compounds from this series were evaluated for their metabolic stability in rat and human microsomes. These compounds showed moderate to good microsomal metabolic stability. The piperidine analogues having methoxypropyl group **2b**, tetrahydropyran group **2e** and 3-aza-bicyclo[3.1.0]hexane analogue having methoxypropyl group **2f**, pyran group **2g** showed higher metabolic stability. Compounds **2b** and **2g**, which showed good metabolic stability, were evaluated in rat pharmacokinetics (PK) studies. Both the compounds showed poor oral bioavailability suggesting lack of correlation between microsomal stability and oral exposures in rats. The brain penetration ($C_b/C_p$) however had substantially improved (1.8 to 2) as against earlier reported Series 1 compound **5a** (0.31).[19] The 1,3,4-oxadiazole derivatives were not evaluated in PK experiments owing to their poor *in vitro* potency.

Given the improvement in brain penetration in Series 2 compounds, the scope of SAR was further expanded by synthesizing and evaluating a series of isopropylimidazo[1,2-*a*]pyridine derivatives (Series-3 , Table 2). Most of the piperidine ring (**$B_1$**) compounds (**3a-3h**) showed potent *in vitro* potency at 5-$HT_4$R ($EC_{50}$ <10 nM). Compounds with *N*-substituted 3-aza-bicyclo[3.1.0]hexane rings (**3i-3m**) however were 2-10 fold less potent (Compounds **3a** vs **3i**; **3b**

vs **3j**). During the course of this research work, a publication[28] from Pfizer reported the formation of a cyclized oxazolidine metabolite of PF-04995274 which was reported as unique to human and was not observed in animal studies. Given the partial structural similarity i.e., tert-hydroxyl group on piperidine *N*-substituents of compounds **3f**, **3g** and **3h** to PF-04995274, theoretically these compounds may also have potential to form such unique metabolites. Though these compounds showed potent *in vitro* activity at 5-$HT_4$R, no additional explorations were carried out. The rat pharmacokinetic studies of compounds **3b** and **3c** showed high clearance, poor exposures and poor bioavailability, an observation akin to Series 2 compounds. Compound **3c** based on its high *in vitro* potency and high brain penetration, was evaluated in the long-term memory deficit assay the Novel Object Recognition Test (NORT) to see the translation of *in vitro* activity into *in vivo* efficacy. The NORT[29,30] has been regarded as a spontaneous Delayed-Non-Matching-to-Sample (DNMS) test. The test is based on a spontaneous behavior. The main assumption at the base of this test is that access to novelty (object or environment) can elicit approach behaviors in animals. NORT doesn't involve reference memory components, thus it can be considered a "pure" recognition memory test and a valid task to assess working memory. Also the test doesn't involve positive or negative reinforces (food or electric shocks) and this makes NORT comparable to memory tests currently used in humans. These advantages make NORT a quick and simple to be implemented and, therefore, it has been widely used for assessing mild cognitive impairment in pre-clinical research. This apparent 'unconditioned preference' for novelty has been used in the NORT in order to study memory functions, assessing the ability of animals to recognize a novel object in a familiar environment. Although this model has face and predictive validity for memory deficits, the percentage of translational success from rat to human is poor till date. In this experiment, the rats treated with **3c** at 0.3 and

1 mg/kg, *p.o.* spent significantly more time exploring the novel object than the familiar object in the test phase, i.e., the rats remembered the familiar object even after 24 h time gap. However, the vehicle treated rats spent almost equal time in exploring the novel object as well as the familiar object forgetting the familiar object after 24 h time gap (Figure 3). Encouraged by these results, further modification of the chemical scaffold was done with the aim to obtain compounds with good oral bioavailability. Chloro substitution was brought in the 3-position of imidazo[1,2-*a*]pyridine to obtain compounds **3n** and **3o** and carbon (C-3) was replaced with nitrogen atom to obtain triazolo compounds **3p** and **3q**. However, these modifications resulted in very weak potency (**3q**) or complete loss of potency (**3n-3p**). However, the microsomal metabolic stability of these compounds has increased substantially. We hypothesized that this improvement in microsomal metabolic stability is probably due to blocking of the high electron density 3-position of imidazo[1,2-*a*]pyridine. However, the 1,3,4-oxadiazole derivatives, **3r** and **3s** showed weak affinity towards 5-$HT_4$R with favorable metabolic stability.

As attempts to improve the metabolic stability by structural modifications resulted in less potent or inactive compounds, further SAR was carried out by synthesizing pyrazolo[1,5-*a*]pyridine derivatives (Series 4, Table 3). As it was evident from the results shown in Table 3, the compounds having piperidine (**$B_1$**) with *N*-substitutions like methoxypropyl (**4a**), pyranylmethyl (**4b**) groups were more potent as compared to isopropyl group (**4c**). The corresponding *N*-substituted 3-aza-bicyclo[3.1.0]hexane derivatives (**4d-4f**) were less potent, similar to Series 2 and Series 3 compounds. Most of the evaluated compounds were extensively metabolized both in rat and human liver microsomes. Based on the availability of resources, compounds **4b** and **4c** have been explored in pharmacokinetics (PK) studies. Both the compounds showed poor oral bioavailability while **4c** showed poor brain penetration as well. In

an attempt to increase the microsomal metabolic stability, 3-chloro-pyrazolopyridine derivatives (**4g-4i**) and triazolopyridine derivatives (**4j-4l**) were synthesized. However, these compounds did not show any activity towards 5-$HT_4$R. In line with earlier observations, the metabolic stability of 3-chloro-pyrazolopyridine derivatives **4g-4i** was moderately improved whereas the metabolic stability of triazolopyridine derivatives **4k-4l** was increased substantially. The 1,3,4-oxadiazole derivatives (**4m-4o**) however, showed potent *in vitro* activity and also showed improved metabolic stability. Compounds **4m, 4n** and **4o** showed good oral bioavailability; Compounds **4n** and **4o** showed improved brain penetration. However, the *N*-isopropyl substituted derivative **4m** showed poor brain penetration (Table 3). Compounds **4n** and **4o** were evaluated in efficacy models of cognition. In NORT, compound **4o** showed robust efficacy at 3 and 10 mg *p.o*. whereas, compound **4n** showed efficacy at 1 and 10 mg *p.o*. (Figure 3). The reference compound RS-67333 a 5-$HT_4$R partial agonist[17] showed efficacy at 0.3 and 1.0 mg/kg *i.p*. In a separate experiment, the observed activity of **4o** in NORT was blocked by administering the selective 5-$HT_4$R antagonist GR-125487[31,32] giving ample evidence that the efficacy observed may be arising from its agonistic activity at 5-$HT_4$R (Figure 4).

In order to further substantiate the finding that the observed efficacy is arising from 5-$HT_4$R agonism, compounds **4n** and **4o** were evaluated for their receptor occupancy (R.O.).[33] Both the compounds showed significant R.O. showing absolute $ED_{50}$ values of 8.47 and 6.15 mg/kg, *p.o*., respectively (Figure 5) suggesting the *in vivo* efficacy observed for these compounds may be mediated through 5-$HT_4$R. The partial agonist from Pfizer, PF-04995274 showed dose dependent R.O. with $ED_{50}$ value of 0.017 mg/kg, *s.c*. which is comparable with the reported value (0.008 mg/kg, *s.c*.).[34]

The protein binding experiment was done in rat, dog and human liver microsomes to ascertain the free fraction available for **4n** and **4o**. Compound **4n** showed 26% free fraction in rat plasma and 14% in brain homogenate whereas compound **4o** showed 17% free fraction in rat plasma and 24% in brain homogenate. The percentage free fraction for both compounds in plasma was similar in other species like dog and human. Both **4n** and **4o** showed minimal inhibition of cytochrome P450 3A4 and 2D6 enzymes and showed absolute $IC_{50}$ values of >10 μM. Compound **4o** based on its favorable *in vitro* affinity, higher oral exposures, dose dependent R.O., robust efficacy in NORT and significant free fraction in brain was selected for further evaluation in microdialysis studies. It is known in the literature that 5-$HT_4$R agonists improve memory formation by enhancing the synaptic release of acetylcholine (the neurotransmitter responsible for cognition process) in the brain which would in turn enhance the cholinergic transmission.[8] The microdialysis experiments showed that compound **4o** increased acetylcholine levels dose-dependently in frontal cortex upon oral dosing at 3 and 10 mg/kg (Figure 6). The 5-$HT_4$R partial agonist PRX-03140 was used as a positive control. At 1 mg/kg, *i.p.* PRX-03140 produced about 254% increase from pre-dose levels. The increase in acetylcholine levels by PRX-03140 was in agreement with the literature reports.[35]

The 5-$HT_4$R agonists have the ability to shift the equilibrium of amyloid precursor protein (APP) processing from amyloidogenic to non-amyloidogenic form showing disease modifying potential.[36] The APP processing shifts equilibrium from amyloidogenic to non-amyloidogenic form by generating sAPPα which is reported to provide neuroprotective effect.[7,37,38] In this experiment, 5-$HT_4$R agonist prucalopride was used as a positive control. Treatment with prucalopride (10 mg/kg, *s.c.*) produced 65% increase above control levels. These results are in agreement with the literature.[37] Increase in sAPPα levels produced by compound **4o** (1-10

mg/kg, *s.c.*) were higher than the effects showed by prucalopride (10 mg/kg, *s.c.*). The increase in cortical sAPPα levels in mice demonstrated that compound **4o** may have disease modifying potential (Figure 7).

Compounds **4n** and **4o** showed comparable potencies at 5-$HT_4$R isoforms (5-$HT_4$D, 5-$HT_4$E and 5-$HT_4$L) with $EC_{50}$ values ranging from 6 to 62 nM. Compounds **4n** and **4o** showed weak propensity to inhibit hERG channel in electrophysiology patch clamp assay and the $IC_{50}$ was found to be 2.3 μM with 76% $I_{max}$ and 1.9 μM with 100% $I_{max}$ at 10 μM concentration respectively. Compounds **4n** and **4o** showed excellent selectivity against 5-HT receptors (5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$, 5-$HT_7$), Adrenergic receptors ($Ad\alpha_{1B}$, $Ad\alpha_{2C}$), Adenosine receptor $A_{2A}$, Cannabinoid receptor ($CB_1$ and $CB_2$), Dopamine ($D_1$, $D_{2S}$, $D_3$ and $D_5$), Histamine ($H_1$ and $H_3$), Muscarinic receptors ($M_1$, $M_2$, $M_3$, $M_4$ and $M_5$) and serotonin transporter. Both the compounds did not show any significant binding at highest tested concentration of 10 μM towards GPCRs, ion channels and transporters (See Supporting Information). Compound **4o** showed partial agonist activity at 5-$HT_2$B receptor when evaluated in rat fundus assay[39] (See Supporting Information). Based on the panel of selectivity assessment involving majority of GPCRs which are responsible for cognition, we hypothesized that the compound **4o** selectively modulates 5-$HT_4$R. Further evaluation of compound **4o** in additional efficacy models, receptor mapping and extended safety will be done in due course of time.

## CONCLUSIONS

In summary, starting from a poorly brain penetrant **5a**, effecting successive modifications in the position of nitrogen atom, position of isopropyl group, position of carboxamide group in heteroaromatic ring resulted in identification of a series of pyrazolo[1,5-*a*]pyridine derivatives

represented by compound **4o**. The lead compound **4o** is a potent and selective 5-$HT_4$R partial agonist with high oral exposures and good brain penetration. *In vivo* R.O. studies demonstrated a clear relationship between the *in vitro* affinity, free brain exposure, *in vivo* efficacy and R.O. . We have also demonstrated in this report that the compounds **3c**, **4n** and **4o** improved long-term memory deficits in NORT, a finding that is consistent with previous reports. The attenuation of compound **4o** efficacy in NORT by selective 5-$HT_4$R antagonist GR-125487 and dose dependent 5-$HT_4$ R.O. provides ample evidence that the cognitive effects of compound **4o** was mediated through 5-$HT_4$R. Compound **4o** increased neurotransmitter ACh release in prefrontal cortex, a finding that is consistent with previous reports. Compound **4o** increased neuroprotective sAPPα levels thus offering disease modifying therapy. A safety assessment of this compound is in progress to ascertain the therapeutic margins between efficacious concentrations and safety findings.

## EXPERIMENTAL SECTION

Unless stated otherwise, all reagents and solvents were purchased from common commercial suppliers and were used without further purification. $^{1}$H-NMR spectra were recorded at 400 MHz and $^{13}$C-NMR at 100 MHz on a Bruker NMR spectrometer instrument (Fallanden, Switzerland). All $^{1}$H NMR shifts are reported in δ units (ppm) relative to the signals for chloroform (7.27 ppm), DMSO (2.50 ppm), and MeOH (3.31 ppm). All coupling constants (*J* values) are reported in hertz (Hz). NMR abbreviations are as follows: bs, broadened singlet; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and dd, doublet of doublets. Thin layer chromatography (TLC) was performed on Merck silica gel 60 $F_{254}$ plates. Electrospray ionization mass spectra were recorded on API 4000 triple quadrupole instrument (MDS-SCIEX, Concord, Ontario, Canada).

Infrared spectra were recorded on KBr disc and in solid state using Perkin-Elmer model 1600 FT-IR spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). Elemental analyses were carried out in an 'Elementar' GmbH, Vario micro cube instrument and the 'analyses indicated by the symbols of the elements were within ± 0.4 % of the theoretical values'. Column chromatography was performed using 100-200 mesh silica gel. HPLC purity of the final compounds was done using Agilant systems (Model-1100 series). DSC was recorded on Waters DSC Q100 instrument. Melting points of synthesized compounds were determined using Electro Derman open capillary apparatus and are uncorrected. The reference standards PRX-03140, RS-67333, PF-04995274, GR-125487 prucalopride and others were synthesized and characterized in-house using respective reported procedures. All final compounds were determined to have a purity of >95% by one of the aforementioned methods unless stated otherwise.

**Synthesis of 2-Methyl-1-(pyridin-2-yl)-1-propanol 2**: To a stirred solution of 2-bromopyridine, **1** (13 g, 82.3 mmol) in dry THF (150 mL) cooled at –78 °C, *n*-BuLi (45.0 mL, 1.6 M in hexane) was added drop-wise over a period of 30 minutes. Isobutyraldehyde (15.0 mL, 153.0 mmol) was added over a period of 15 minutes and after stirring the reaction mixture for 15 minutes at this temperature, it was gradually warmed to r.t. and was stirred for 2 h. The reaction mixture was then cooled to 0 °C and was quenched by adding ice water followed by addition of EtOAc. The two layers were separated and the aqueous layer was extracted again with EtOAc. The combined organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to afford crude product which was purified by silica gel column chromatography to obtain 6.1 g of the above titled compound **2** in 47% yield.$^1$H - NMR (400 MHz, $CDCl_3$): δ 8.55 (d, $J$ = 4.7 Hz, 1H), 7.69 (t, $J$ = 7.8 Hz, 1H), 7.19 (d, $J$ = 2.2 Hz, 2H), 4.19 (d, $J$ = 5.8 Hz, 1H), 2.15-2.05 (m, 1H), 1.02 (d, $J$ = 6.8 Hz, 3H), 0.81 (d, $J$ = 6.4 Hz, 3H); Mass (m/z): 152.1 $(M+H)^+$.

**Synthesis of 2-Methyl-1-(pyridin-2-yl)-1-azidopropane 3**: To a stirred solution of compound **2** (5.5 g, 36.3 mmol), triethylamine (10.0 mL, 72.6 mmol) in dry DCM (150.0 mL) cooled at 0 °C was added methanesulfonyl chloride (3.0 mL, 36.3 mmol) over a period of 15 minutes. After stirring the reaction mixture for another 30 minutes, it was diluted with water (50.0 mL), DCM (50 mL) and the two layers were separated. The aqueous layer was further extracted with DCM (2x50 mL). The combined organic layer was washed with water (150 mL), brine (150 mL), dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain the mesylated intermediate which was dissolved in DMF (66.0 mL) and $K_2CO_3$ (10.0 g, 72.6 mmol) followed by $NaN_3$ (3.5 g, 57.3 mmol) were added. The reaction temperature was gradually raised to 80 °C and the reaction mass was maintained at this temperature for 2 h before bringing it to r.t. The reaction mixture was diluted with water (100 mL) and EtOAc (150 mL) and the two layers were separated. The organic layer was further washed with water followed by brine solution, dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain the titled azido 2-methyl-1-(pyridin-2-yl)-1-azidopropane **3** (6.0 g) in 99% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 8.60 (d, $J$ = 4.4 Hz, 1H), 7.73 (t, $J$ = 7.8 Hz, 1H), 7.30 (d, $J$ = 7.8 Hz, 1H), 7.24 (dd, $J$ = 4.4, 7.8 Hz, 1H), 4.26 (d, $J$ = 7.3 Hz, 1H), 2.30-2.10 (m, 1H), 1.0 (d, $J$ = 6.6 Hz, 3H), 0.88 (d, $J$ = 6.7 Hz, 3H); Mass (m/z): 177.2 $(M+H)^+$.

**Synthesis of 2-Methyl-1-(pyridin-2-yl)-1-aminopropane 4**: To a stirred solution of compound **3** (6.0 g, 36.3 mmol) in THF (68.0 mL) at r.t., triphenylphosphine (11.0 g, 44.6 mmol) followed by water (3.1 mL, 185.0 mmol) was added. After 16 hours stirring at r.t., the volatiles were removed under reduced pressure and the crude reaction mixture was purified by silica gel column chromatography to obtain compound **4** (3.83 g) in 70% yield.$^1$H - NMR (400 MHz, $CDCl_3$): δ 8.55 (d, $J$ = 2.4 Hz, 1H), 7.64 (t, $J$ = 7.6 Hz, 1H), 7.26 (d, $J$ = 1.8 Hz, 2H), 7.13 (t, $J$ =

6.0 Hz, 1H), 3.69 (d, *J* = 1.2 Hz, 1H), 2.07-2.01 (m, 1H), 0.95 (d, *J* = 1.6 Hz, 3H), 0.79 (d, *J* = 3.5 Hz, 3H); Mass (m/z): 151.1 $(M+H)^+$.

**Synthesis of Ethyl *N*-[2-methyl-1-(pyridin-2-yl)-propyl]-oxalamate 5**: To a stirred solution of compound **4** (3.8 g, 25.3 mmol) and triethylamine (10.0 mL, 50.6 mmol) in solvent DCM (50 mL) cooled at 0 °C, ethyl chlorooxoacetate (3.0 mL, 25.3 mmol) was added over a period of 10 minutes. After stirring the reaction mixture at this temperature for 1h, it was diluted with DCM (50 mL), washed with water (50 mL), dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain compound **5** (5.52 g) in 87% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 8.57 (d, *J* = 3.9 Hz, 1H), 8.28 (d, *J* = 7.2 Hz, 1H), 7.68 (t, *J* = 12.2 Hz, 1H), 7.19 (d, *J* = 7.4 Hz, 2H), 4.88 (t, *J* = 7.2 Hz, 1H), 4.35 (q, 2H), 2.23-2.15 (m, 1H), 1.37 (t, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H); Mass (m/z): 251.1 $(M+H)^+$.

**Synthesis of Ethyl 1-isopropyl imidazo[1,5-*a*]pyridine-3-carboxylate 6**: To a stirred solution of compound **5** (5.5 g, 22.0 mmol) in 1,2-dichloroethane (50.0 mL) at r.t., $POCl_3$ (20.0 mL) was added drop-wise over a period of 15 minutes. The reaction mixture was then heated to 130 °C for 72 h. The volatiles were removed under reduced pressure to obtain a crude mass which was neutralized with $NaHCO_3$ solution and extracted with EtOAc. The combined organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography to obtain compound **6** (3.58 g) in 70% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 9.32 (d, *J* = 7.1 Hz, 1H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.00 (t, *J* = 6.6 Hz, 1H), 6.88 (t, *J* = 6.7 Hz, 1H), 4.58-4.50 (m, 2H), 3.47-3.39 (m, 1H), 1.43 (d, *J* = 6.6 Hz, 6H), 1.32 (t, 3H); Mass (m/z): 233.1 $(M+H)^+$.

**Synthesis of Ethyl 2-isopropyl-imidazo[1,2-*a*]pyridine-8-carboxylate 8**: To a stirred solution of ethyl 2-aminonicotinate **7** (0.8 g, 3.44 mmol) in ethanol (4.72 mL), at r.t., 1-chloro-3-methylbutan-2-one (6.9 g, 41.2 mmol) was added. The reaction mixture was stirred at 95 °C for 4 h. The solvent was removed under reduced pressure and the crude reaction mixture was purified by silica gel column chromatography to obtain compound **8** (0.45 g) in 58% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 8.22 (d, *J* = 6.6 Hz, 1H), 7.87 (d, *J* = 7.1 Hz, 1H), 7.40 (s, 1H), 6.79 (t, *J* = 6.9 Hz, 1H), 4.50 – 4.45 (q, 2H), 3.26–3.19 (m, 1H), 1.39 (d, *J* = 6.8 Hz, 6H); Mass (m/z): 233.2 $(M+H)^+$.

**Synthesis of 3-Methyl-1-pyridin-2-yl-2-butanone 10**: To a stirred solution of 2-picoline **9** (20.0 g, 214.7 mmol) in dry THF (860.0 mL) cooled at −78 °C, *n*-BuLi (1.6 M in hexanes, 134.2 mL, 214.7 mmol) was added over a period of 15 minutes. After the reaction mixture was stirred for 1 hour at this temperature, isobutyryl chloride (25.2 g, 236.2 mmol) was added at once and the reaction mass was stirred for another 30 minutes. The reaction mass was quenched by adding saturated ammonium chloride solution. The two layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous $Na_2SO_4$ and the volatiles were removed under reduced pressure to obtain a crude mass which was purified by silica gel column chromatography to obtain the titled compound 10 (5.4 g) in 13% yield.$^1$H - NMR (400 MHz, $CDCl_3$): δ 8.54 (d, *J* = 4.3 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 7.16 (d, *J* = 6.7 Hz, 1H), 3.96 (s, 2H), 2.86-2.77 (m, 1H), 1.13 (d, *J* = 6.4 Hz, 6H); Mass (m/z): 164.33 $(M+H)^+$.

**Synthesis of 2-Isopropyl pyrazolo[1,5-*a*]pyridine 11**: To a stirred solution of compound **10** (3.3 g, 3.44 mmol) in $CHCl_3$ (67.0 mL), cooled at 0 °C, *O*-mesitylenesulfonylhydroxylamine

(4.35 g, 20.2 mmol) was added. The reaction mass stirred at r.t. for 1 hour, upon which TLC revealed completion of the reaction, the volatiles were removed and the crude mass was purified by silica gel column chromatography to obtain above titled compound **11** (1.0 g) in 31% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 8.38 (d, *J* = 8.8 Hz, 1H), 7.4 (d, *J* = 8.8 Hz, 1H), 7.04 (t, *J* = 8.2 Hz, 1H), 6.66 (t, *J* = 6.4 Hz, 1H), 6.3 (s, 1H), 3.21 (m, 1H), 1.37 (d, *J* = 6.8 Hz, 6H); Mass (m/z): 161.1 $(M+H)^+$.

**Synthesis of compounds 13a**, **13b** and **13c by *N*-alkylation with alkyl halides**: Followed the procedure as reported in our own patent publication *WO2013042135* with minor modifications. To a stirred solution of ethyl isonipecotate **12** (1.0 mmol) in acetonitrile (2.0 mL), at r.t., was added $Cs_2CO_3$ (2.2 mmol) and alkyl halide (1.1 mmol). The reaction mass was gradually heated to reflux temperature and stirred at this temperature for 4-12 h until all starting material gets consumed. The reaction mixture was cooled to r.t., and was filtered through a small pad of celite. The filtrate was evaporated under reduced pressure which afforded the *N*-alkylated ethyl isonipecotates **13a**, **13b** and **13c** respectively in quantitative yields.

**Synthesis of compounds 14a**, **14b** and **14c**: Followed the procedure as reported in our own patent publication *WO2013042135* with minor modifications. To a stirred solution of *N*-alkylated ethyl isonipecotate (**13a**, **13b** or **13c,** 1.0 mmol) in THF (2.0 mL), and water (2.0 mL) cooled at 0 °C was added $LiOH.H_2O$ (1.1 mmol). After stirring for 30 minutes at this temperature, the reaction mass was brought to r.t., and stirred for 6-12 h until all ester gets hydrolyzed. Upon completion of the reaction, the reaction mass was diluted with EtOAc and the two layers were separated. The aqueous layer was further extracted with EtOAc and the resultant aqueous layer evaporated under reduced pressure which afforded *N*-alkylated lithium carboxylates **14a**, **14b** or

**14c** in quantitative yields respectively which were used in the next step without further purification.

**Synthesis of 1-Isopropyl-imidazo[1,5-*a*]pyridine-3-carboxylic acid hydrazide 15**: To a stirred solution of **6** (0.23 g, 1.0 mmol) in EtOH (10.0 mL) at r.t., hydrazine hydrate (2.0 mL) was added. The reaction mixture was heated to reflux for 8 h until all ester got consumed. The volatiles were removed under reduced pressure to obtain a crude mass which was triturated with hexane followed by ether which afforded hydrazide **15** as brown color solid (0.21 g) in 90% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 9.36 (s, 1H), 9.28 (d, $J$ = 7.0 Hz, 1H), 7.78 (d, $J$ = 8.9 Hz, 1H), 6.99-6.94 (m, 2H), 4.47 (s, 2H), 3.39-3.30 (m, 1H), 1.32 (d, $J$ = 6.9 Hz, 6H); Mass (m/z): 219.2 $(M+H)^+$.

**Synthesis of 2-Isopropyl-imidazo[1,2-*a*]pyridine-8-carboxylic acid hydrazide 16**: Following the procedure followed for preparing compound **15**, compound **16** was obtained as dark brown color solid in 85% yield.$^1$H - NMR (400 MHz, $CDCl_3$): δ 11.39 (bs, 1H), 8.17 (d, $J$ = 6.6 Hz, 1H), 8.13 (d, $J$ = 7.0 Hz, 1H), 7.38 (s, 1H), 6.90 (t, $J$ = 6.9 Hz, 1H), 4.29 (d, $J$ = 3.7 Hz, 2H), 3.14 (m, 1H), 1.38 (d, $J$ = 6.8 Hz, 6H); Mass (m/z): 219.2 $(M+H)^+$.

**Synthesis of methyl 2-isopropylpyrazolo[1,5-*a*]pyridine-7-carboxylate 17**: To a stirred solution of acid $\mathbf{A_3}$ (0.23 g, 1.1 mmol) in MeOH (5.0 mL), cooled at 0 °C, drop-wise addition of thionyl chloride (0.2 mL). The reaction temperature was gradually raised to 65 °C, and the reaction mixture was stirred at this temperature for 4 h. The volatiles were removed under reduced pressure and the crude mass was diluted with DCM and aqueous $NaHCO_3$ solution. The two layers were separated, the organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain ester **17** (0.25 g) in 100% yield. $^1$H - NMR (400

MHz, $CDCl_3$): δ 7.63 (d, J = 8.7 Hz, 1H), 7.40 (d, *J* = 6.8 Hz, 1H), 7.07 (t, *J* = 7.3 Hz, 1H), 6.46 (s, 1H), 4.0 (s, 3H), 3.34 (m, 1H), 1.38 (d, *J* = 6.7 Hz, 6H); Mass (m/z): 219.2 $(M+H)^+$.

**Synthesis of 2-Isopropylpyrazolo[1,5-*a*]pyridine-7-carboxylic acid hydrazide 18**: Following the procedure used for preparing compound **15**, compound **18** was obtained as dark brown color solid in 95% yield.$^1$H - NMR (400 MHz, DMSO): δ 11.4 (bs, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.69 (d, *J* = 6.9 Hz, 1H), 7.37 (t, *J* = 7.8 Hz, 1H), 6.68 (s, 1H), 4.95 (bs, 2H), 3.28-3.20 (m, 1H), 1.35 (d, *J* = 6.8 Hz, 6H); Mass (m/z): 219.2 $(M+H)^+$.

**Synthesis of *N*-[*N*-tert-butyloxycarbonyl-3-azabicyclo[3.1.0]hexane-6-yl methyl]1-isopropyl imidazo[1,5-*a*]pyridine-3-carboxamide 19b**: To the stirred solution of acid $\mathbf{A_1}$ (600.0 mg, 2.94 mmol) in DCM (11.8 mL) cooled at 0 °C, diisopropylethyl amine (0.77 mL, 4.41 mmol), tert-butyl 6-aminomethyl-3-aza-bicyclo[3.1.0]hexane-3-carboxylate $\mathbf{B_2}$ (624.0 mg, 2.94 mmol) and TBTU (944.0 mg, 2.94 mmol) were sequentially added. The reaction temperature was gradually brought to r.t. and the reaction mixture was stirred for 16 h before being diluted with DCM and water. The two layers were separated, the aqueous layer was extracted once with DCM and the combined organic layer was washed with brine solution. The resultant organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was evaporated under reduced pressure. The crude mass was purified by silica gel column chromatography to obtain above titled compound **19b** (1.03 g) in 85% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 9.38 (d, *J* = 7.1 Hz, 1H), 7.56 (d, *J* = 9.9 Hz, 1H), 7.44 (t, *J* = 5.2 Hz, 1H), 6.86 (t, *J* = 9.9 Hz, 1H), 6.75 (t, *J* = 6.8 Hz, 1H), 3.67 (d, *J* = 6.7 Hz, 2H), 3.39 - 3.36 (m, 4H), 3.09 – 3.07 (m, 1H), 1.42 (s, 9H), 1.39 - 1.37 (m, 8H), 1.03-0.97 (m, 1H); Mass (m/z): 399.3 $(M+H)^+$.

**Synthesis of *N*-[*N*-3-azabicyclo[3.1.0]hexane-6-yl methyl]1-isopropyl imidazo[1,5-*a*]pyridine-3-carboxamide hydrochloride 20b:** To the stirred solution of Boc protected amide derivative **19b** (1.0 g, 2.51 mmol) in isopropanol (5.0 mL) cooled at 0 °C, a solution of dry HCl in IPA (5.0 mL) was added. The contents were stirred for 16 h at r.t. before the volatiles were removed under reduced pressure. The HCl salt thus obtained was triturated with several portions of ether to obtain the above titled compound **20b** (630.0 mg) as white solid in 98% yield. m.p. 172-174 °C. $^1$H - NMR (400 MHz, DMSO): δ 9.55 (bs, 1H), 9.35 (d, *J* = 7.0 Hz, 1H), 8.95 (bs, 1H), 8.59 (t, *J* = 5.6 Hz, 1H), 7.83 (d, *J* = 9.0 Hz, 1H), 7.01 (t, J = 8.4 Hz, 1H), 6.95 (t, *J* = 6.8 Hz, 1H), 3.43 – 3.41 (m, 1H), 3.27 – 3.19 (m, 6H), 1.77 (s, 2H), 1.35 (d, *J* = 6.9 Hz, 7H); Mass (m/z): 299.2 $(M+H)^+$.

**Synthesis of 1-Isopropyl imidazo[1,5-*a*]pyridine-3-carboxylic acid $A_1$**: To a stirred solution of compound **6** (1.56 g, 6.7 mmol) in a mixture of ethanol and water (1:1, 20 mL), NaOH (0.3 g, 7.38 mmol) was added and the reaction mixture was heated to reflux for 2 hours. The volatiles were removed under reduced pressure to one third of its volume, diluted with water, extracted with solvent ether. The aqueous layer was acidified with con. HCl to pH 4-5 and extracted the acidified aqueous layer with mixture of methanol and DCM (1:9). The combined organic layer was dried on anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain acid $\mathbf{A_1}$ (1.33 g) in 97% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 9.20 (d, *J* = 7.0 Hz, 1H), 7.86 (d, *J* = 8.9 Hz, 1H), 7.09 (t, *J* = 6.6 Hz, 1H), 7.02 (t, *J* = 6.7 Hz, 1H), 3.45-3.36 (m, 1H), 1.30 (d, *J* = 6.5 Hz, 6H); Mass (m/z): 205.2 $(M+H)^+$.

**Synthesis of 2-Isopropyl-imidazo[1,2-*a*]pyridine-8-carboxylic acid $A_2$**: To a stirred solution of compound **8** (0.44 g, 2.0 mmol) in a mixture of THF and water (1:1, 7.2 mL), cooled at 0 °C,

NaOH (83.4 mg, 2.2 mmol) was added. The reaction mixture temperature was slowly raised to r.t. and after 3 hours of stirring, the TLC revealed complete consumption of ester. The reaction mass was diluted with ether and the two layers were separated. The aqueous layer was acidified with 1N HCl to pH 4-5and was extracted with DCM. The combined organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain acid **$A_2$** (380.0 mg) in 98% yield.$^1$H - NMR (400 MHz, DMSO): δ 8.76 (d, *J* = 6.5 Hz, 1H), 7.96 (d, *J* = 6.8 Hz, 1H), 7.94 (s, 1H), 7.13 (t, *J* = 6.2 Hz, 1H), 2.95 – 2.93 (m, 1H), 1.28 (d, *J* = 6.4 Hz, 6H); Mass (m/z): 205.3 $(M+H)^+$.

**Synthesis of 2-Isopropylpyrazolo[1,5-*a*]pyridine-7-carboxylic acid $A_3$**: To a stirred solution of compound **11** (0.5 g, 3.1 mmol) in dry THF (12.0 mL), cooled at −78 °C, *n*-BuLi (1.6 M in hexanes, 2.0 mL, 3.2 mmol) was added. The reaction mass was stirred at the same temperature for another hour before being quenched by dry ice (solid $CO_2$). The reaction mixture was diluted with water and EtOAc and the two layers were separated. The aqueous layer was acidified with 1N HCl to pH 2-3 and extracted with chloroform. The combined organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain acid **$A_3$** (35.0 mg) in 55% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 15.2 (s, 1H), 7.88 (d, *J* = 7.0 Hz, 1H), 7.73 (d, J = 8.7 Hz, 1H), 7.31 (t, *J* = 7.4 Hz, 1H), 6.51 (s, 1H), 3.29-3.22 (m, 1H), 1.40 (d, *J* = 6.8 Hz, 6H); Mass (m/z): 205.2 $(M+H)^+$.

**Synthesis of *N*-[*N*-3-methoxypropyl-3-azabicyclo[3.1.0]hexane-6-yl methyl]1-isopropyl imidazo[1,5-*a*]pyridine-3-carboxamide 2f**. To a stirred solution of deprotected amide derivative **20b** (630.0 mg, 1.88 mmol) in acetonitrile (7.5 mL), $K_2CO_3$ (390.0 mg, 2.82 mmol) and 1-bromo-3-methoxypropane (380.0 mg, 2.5 mmol) were added. The reaction mass was heated to reflux for 7 h. The volatiles were removed under reduced pressure. The crude product

was purified by silica gel column chromatography to obtain the desired compound **2f** (620.0 mg) as off-white color gummy liquid in 67% yield. $^1$H - NMR (400 MHz, $CDCl_3$): δ 9.40 (d, *J* = 7.2 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.37 (bs, 1H), 6.83 (t, *J* = 8.0 Hz, 1H), 6.72 (t, *J* = 4.0 Hz, 1H), 3.39 (t, *J* = 8.0 Hz, 2H), 3.25 (m, 1H), 3.27 (s, 3H), 3.30 (t, *J* = 4.0 Hz, 2H), 3.06 (d, *J* = 8.0 Hz, 2H), 2.45 (t, *J* = 8.0 Hz, 2H), 2.30 (d, *J* = 8.0 Hz, 2H), 1.97-1.90 (m, 1H), 1.72-1.65 (m, 2H), 1.49-1.43 (m, 1H), 1.47-1.43 (m,1H), 1.39 (d, *J* = 8.0 Hz, 6H); Mass (m/z); 371.3 $(M+H)^+$. δ 160.2, 139.2, 129.1, 127.2, 124.6, 120.3, 117.4, 114.7, 69.1, 57.5, 55.8, 54.4, 42.3, 39.7, 26.6, 25.7, 21.7, 11.7. HPLC. 99.32%. C,H,N for $C_{21}H_{30}N_4O_2$, calc. C, 68.08, H, 8.16, N, 15.12; found, C, 68.32, H, 8.51, N, 15.32.

**Synthesis of *N*-[*N*-(tetrahydropyran-4-yl-methyl) piperidin-4-yl methyl]-2-isopropyl-imidazo[1,2-*a*]pyridine-8-carboxamide 3c**: Compund **3c** was synthesized following the procuedres as used in synthesis of compound **2f**, in 49% overall yield. $^1$H - NMR (400 MHz, DMSO): δ 10.428 (t, *J* = 5.5 Hz, 1H), 8.67 (d, *J* = 6.5 Hz, 1H), 7.95 (d, *J* = 7.0 Hz, 1H), 7.85 (s, 1H), 7.04 (t, *J* = 7.0 Hz, 1H), 3.87-3.79 (m, 2H), 3.39 (s, 4H), 3.34-3.29 (m, 1H), 2.8 (s, 4H), 1.99 (s, 1H), 1.87-1.80 (m, 2H), 1.67-1.58 (m, 4H), 1.30 (d, *J* = 6.6 Hz, 6H); Mass (m/z): 399.5 $(M+H)^+$. HPLC. 98.12%. C,H,N for $C_{25}H_{36}N_4O_6$, calc. C, 61.46, H, 7.43, N, 11.47; found, C, 61.55, H, 7.89, N, 11.62.

**Synthesis of 2-Isopropyl-7-{5-[1-(3-methoxypropyl)-piperidin-4-yl]-[1,3,4]oxadiazol-2-yl}-pyrazolo[1,5-*a*]pyridine 4n**:

To a stirred solution of lithium 1-(3-methoxypropyl)-piperidine-4-carboxylate **14b** (250.0 mg, 1.2 mmol) in $POCl_3$ (2.0 mL) at r.t., hydrazide **18** (220.0 mg, 1.0 mmol) was added. The reaction mixture temperature was raised to 80 °C-85 °C and was stirred for 10 h. The reaction mixture

was cooled to ice-bath temperature and was quenched by adding 1N NaOH solution. The reaction mass was extracted with $CHCl_3$. The combined organic layer was dried over anhydrous $Na_2SO_4$ and the volatiles were removed under reduced pressure to obtain a crude mass which was purified by silica gel column chromatography to obtain compound **4n** (138.0 mg) in 36% yield. $^1$H - NMR (400 MHz,$CDCl_3$): δ 7.64 (d, *J* = 7.2 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.14 (t, *J* = 7.2 Hz, 1H), 6.49 (s, 1H) 3.44 (t, *J* = 6.4 Hz, 2H), 3.34 (s, 3H), 3.29-3.24 (m, 1H), 3.16-3.11 (m, 1H), 3.02 (t, 2H), 2.49 (t, 2H), 2.20 (t, 2H), 2.12 (t, *J* = 12.0 Hz, 2H), 1.83-1.80 (m, 2H), 1.64-1.54 (m, 2H), 1.39 (d, *J* = 8.0 Hz, 6H); Mass (m/z); 384.2 $(M+H)^+$. C,H,N for $C_{21}H_{29}N_5O_2$, calc. C, 65.77, H, 7.62, N, 18.26; found, C, 65.49, H, 7.37, N, 17.98.

**Oxalate salt formation**: To the stirred solution of above obtained 1,3,4-oxadiazole **4n** (96.0 mg, 0.25 mmol) in isopropanol (4.0 mL) cooled at 0 °C, oxalic acid (23.0 mg, 0.25 mmol) was added and the reaction mass was stirred for 1 h. The volatiles were removed under reduced pressure and the crude salt thus obtained was triturated with several portions of solvent ether to obtain oxalate salt of **4n** in quantitative yield. $^1$H - NMR (400 MHz, DMSO-$d_6$): δ 7.90 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 6.8 Hz, 1H), 7.34 (dd, *J* = 7.5, 8.4 Hz, 1H), 6.68 (s, 1H) 3.50-3.36 (m, 5H), 3.24 (s, 3H), 3.20-3.10 (m, 1H), 3.10-2.95 (m, 4H), 2.40-2.30 (m, 2H), 2.20-2.05 (m, 2H), 1.95-1.85 (m, 2H), 1.32 (t, *J* = 6.8 Hz, 6H); Mass (m/z); 384.3 $(M+H)^+$. $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 168.3, 164.4, 161.6, 159.2, 150.8, 141.3, 123.4, 122.4, 121.0, 115.3, 95.0, 69.0, 57.8, 53.6, 50.2, 30.0, 27.6, 25.9, 23.8, 22.6, 22.5. M.R. 143.8 °C-149.9 °C. HPLC. 99.08%

**Synthesis of 2-Isopropyl-7-{5-[1-(tetrahydropyran-4-ylmethyl)-piperidin-4-yl]-[1,3,4]oxadiazol-2-yl}-pyrazolo[1,5-*a*]pyridine 4o**: Following the procedure used in the synthesis of compound **4n** as depicted above, compound **4o** was prepared (180.0 mg) in 44%

yield using lithium 1-(tetrahydropyran-4-ylmethyl)-piperidine-4-carboxylate **14c** (280.0 mg, 1.2 mmol) and hydrazide **18** (220.0 mg, 1.0 mmol). $^1$H - NMR (400 MHz, CDCl3): δ 8.10 (s, 1H), 8.0 (d, *J* = 7.0 Hz, 1H), 7.86 (d, *J* = 8.32Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 5.03–4.98 (m, 1H), 3.99 (d, *J* = 3.0 Hz, 1H), 3.96 (d, *J* = 3.2 Hz, 1H), 3.42 (t, *J* = 11.2 Hz, 2H), 3.14-3.06 (m, 1H), 2.95 (d, *J* = 10.1 Hz, 2H), 2.22 (d, *J* = 6.9 Hz, 2H), 2.13 – 1.99 (m, 6H), 1.77 (m, 1H), 1.69 (d, *J* = 6.6 Hz, 6H), 1.32-1.22 (m, 2H); Mass (m/z); 410.3 $(M+H)^+$. C,H,N for $C_{23}H_{31}N_5O_2$, calc. C, 67.46, H, 7.63, N, 17.10; found, C, 67.23, H, 7.54, N, 17.47.

**Fumarate salt formation**: To the stirred solution of above obtained 1,3,4-oxadiazole **4o** (103.0 mg, 0.25 mmol) in isopropanol (4.0 mL) cooled at 0 °C, fumaric acid (30.0 mg, 0.25 mmol) was added and the reaction mass was stirred for 1 h. The volatiles were removed under reduced pressure and the crude salt thus obtained was triturated with several portions of solvent ether to obtain fumarate salt of **4o** in quantitative yield. $^1$H - NMR (400 MHz, DMSO-$d_6$ + $D_2O$): δ 8.58 (s, 1H), 8.01 (d, *J* = 8.2, Hz, 1H), 7.96 (d, *J* = 6.9 Hz, 1H), 7.22 (t, *J* = 7.6 Hz, 1H), 6.46 (s, 2H), 4.94 – 4.88 (m, 1H), 3.84-3.80 (m, 2H), 3.50-3.25 (m, 5H), 3.0-2.90 (m, 2H), 2.88-2.80 (m, 2H), 2.35-2.25 (m, 2H), 2.10-1.97 (m, 3H), 1.65-1.60 (m, 2H), 1.58 (d, *J* = 6.6 Hz, 6H), 1.20 – 1.10 (m, 2H); Mass (m/z); 410.0 $(M+H)^+$. $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 169.5, 166.3, 161.6, 159.0, 141.3, 134.2, 123.5, 122.4, 120.9, 115.2, 95.0, 66.8, 63.6, 52.1, 32.1, 31.6, 28.4, 27.5, 22.6. M.R. 205.2 °C-209.6 °C. HPLC. 98.38%.

**Animals and ethics**

All animal care and experiments were carried out according to protocols approved by the Institutional Animal Ethics Committee (IAEC) of Suven Life Science Ltd constituted according

to the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

**Determination of $EC_{50}$ values for 5-$HT_4$R**

The compounds were screened in the cell based reporter gene assay an end point luminescence assay which measures the levels of cAMP inside cells upon activation or inhibition of the receptor, using the luciferase reporter. Serotonin (5-HT) and Luciferin was purchased from Sigma Aldrich (St. Louis, Missouri, USA). T4 DNA Ligase and high fidelity Taq polymerase were procured from Roche (Basel, Switzerland). Superscript Reverse Transcriptase and mammalian expression vector pcDNA3.1 were purchased from Invitrogen (Carlsbad, California, USA). CRE-Luc reporter gene construct was supplied by Stratagene (La Jolla, California, USA). Cell culture media and sera were procured from Invitrogen (Carlsbad, California, USA). All other DNA restriction and modification enzymes were from New England Biolabs (Ipswich, Massachusetts, USA). All other reagents and common chemicals were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Human 5-$HT_4$R cDNA clone was purchased from Origene (Rockville, Maryland, USA). The coding sequence was amplified by PCR using gene specific primers. Amplified DNA was cloned in to mammalian expression vector pcDNA 3.1. The authenticity of the cloned genes was determined by restriction analysis and nucleotide sequencing. CHO cell line was purchased from American Type Culture Collection, Manassas, Virginia, USA. The cells were routinely cultured in Hams F12 medium supplemented with 10% fetal bovine serum at 37 °C in 5% $CO_2$. The cells were transfected with 5-$HT_4$ isoforms along with three-fold excess of CRE-Luc reporter gene using Lipofectamine as recommended by the supplier. Cells were further cultured in the same medium supplemented with 800 μg/ ml G418. Individual colonies were picked up after two weeks of selection and screened for serotonin

induced luciferase activity. Colonies exhibiting maximum serotonin induced luciferase activity were selected and amplified for further investigations. The recombinant CHO cells were plated in 96 well clear bottom white plates (Corning) and cultured overnight as described above. Cells were grown overnight in serum free medium before incubation with compounds to prevent receptor desensitization due to presence of endogenous ligands in the serum.

The reference endogenous agonist serotonin and test compounds in Opti-MEM medium at 11 different test concentrations starting from 10 µM until 0.1 nM in three-fold serial dilutions were incubated with the cells separately in individual wells for 4 hours at 37 ºC in 5 % $CO_2$. Compounds whose potency is less than 1 nM were tested in dose response study whose test concentrations were Starting from 1 µM until 0.01 nM. After incubation, the medium was removed; cells were washed with phosphate buffered saline and lysed in lysis buffer (Tris-10mM (pH-8.0), NaCl-50mM, DTT-1mM, Protease inhibitor cocktail-1X, NP40-0.1%). The luciferase activity was measured in individual wells using luciferin substrate in Victor Light Luminometer Perkin Elmer.

The maximum response produced by each drug was normalized to the 5-HT induced maximum response. Data was analyzed using Graphpad prism software to derive $EC_{50}$ values that correspond to the concentration of agonists required to obtain half-maximal stimulation of adenylyl cyclase. Values were expressed as the geometric mean of two independent experiments performed in triplicates. Reference compound PF-04995274 a reported partial agonist[9] showed partial agonist activity in our 5-$HT_{4e}$ cell based reporter gene assay with an $EC_{50}$ value of 1.3 nM and $E_{max}$ 18% which is very well in agreement with published data of $EC_{50}$ / $E_{max}$ : 0.26 nM / 7%. Some of other reported compounds $EC_{50}$ values were also determined using our assay and

compared with the published data. The $EC_{50}$ values obtained versus reported values tabulated in the supporting information of this manuscript.

**Microsomal Metabolic Stability**

Compounds were incubated with microsomes at 0.5 mg protein /mL in 100 mM phosphate buffer pH 7.4 at 37 °C. Reactions were initiated by addition of NADPH. All incubations were carried out for 30 min. At 30 min, an aliquot of the sample was removed and transferred into 300 μL of acetonitrile. The samples were stored at –20 °C until analysis. Samples were analyzed using LC-MS/MS. Results were expressed as % metabolized at 30 min post incubation.

**CYP 3A4 and 2D6 inhibition**

CYP 3A4 and 2D6 inhibitory potential of test compounds was studied using human liver microsomes. Inhibitory activity was evaluated by incubating the test compound (0.1 – 45 μM) in duplicates for 2 (3A4) and 12 minutes (2D6) with human liver microsomes in presence of isoform-specific substrate (midazolam-3A4 or dextromethorphan-2D6) and NADPH regeneration system. Incubations were terminated by adding ice-cold acetonitrile containing an internal standard and metabolites were quantified using LC-MS/MS. Peak area ratios of analyte versus internal standards were used for calculating $IC_{50}$.

**Pharmacokinetic Study in Rats**

Male Wistar rats (225 ± 25 gm) were used as experimental animals. Three animals were housed in each cage. Two days prior to dosing day, male Wistar rats were anesthetized with isoflurane for surgical placement of jugular vein catheter. Animals were fasted over night before oral dosing (*p.o.*) and food pellets were allowed 2 hours post dosing, whereas during intravenous

dosing food and water were provided *ad libitum*. Three rats were dosed with compounds orally (3 mg/kg) and intravenously (1 mg/kg). Dose formulation was prepared by using water as a vehicle (10 mL/kg for oral and 2 mL/kg for intravenous dosing). At each time point blood was collected through jugular vein and immediately replenished with an equivalent volume of normal saline from freely moving rats. Collected blood was transferred into a labeled eppendorf containing 10 µL of heparin as anticoagulant. The time points for blood samples collection was; pre dose, 0.08 (only i.v.), 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours post dose (n=3). The collected blood was centrifuged at 4000 rpm for 10 minutes. Plasma was prepared and stored at −70 °C until analysis. The test compound concentrations were extracted from plasma samples by protein precipitation using four volumes of acetonitrile (v/v) containing predetermined internal standard. The precipitants are centrifuged (at 11292xg and 10 °C for 10 min) and supernatant is transferred and injected in LC-MS/MS. The analytes of interest are quantified using suitable multiple reaction monitoring mode in the range of 2-2000 ng/mL or ng/g against set of calibration standards and quality control samples. Study samples were analyzed using calibration samples in the batch and quality control samples spread across the batch. Pharmacokinetic parameters were determined by using standard non-compartmental analysis (Phoenix WinNonLin® 6.2 version or higher software) using linear trapezoidal with linear interpolation method.

**Rodent Brain Penetration Study**

Male Wistar rats (225 ± 25 gm) were used as experimental animals. Three animals were housed in each cage. Animals were given water and food *ad libitum* throughout the experiment and maintained on a 12 h light/dark cycle. Brain penetration was determined in discrete manner in rats. The compounds were formulated in water and administered orally at 10 mg/kg (free base

equivalent). Blood samples were removed via, cardiac puncture by using isoflurane anesthesia at one hour post dose. The animals were sacrificed to collect the brain tissue. Plasma was separated and brain samples were homogenized and stored at −70 °C until analysis. The concentrations of the compound in plasma and brain were determined using LC-MS/MS method. The test compound concentrations are extracted from brain homogenate by protein precipitation using four volumes of acetonitrile (v/v) containing predetermined internal standard. The precipitants are centrifuged (at 11292xg and 10 °C for 10 min) and supernatant is transferred and injected in LC-MS/MS. The compounds were quantified in the calibration range of 1-2000 ng/mL in plasma and brain homogenate. Study samples were analyzed using calibration samples in the batch and quality control samples spread across the batch. Extent of brain to plasma ratio was calculated ($C_b/C_p$).

**Novel Object Recognition Task**

Male Wistar rats 10-12 weeks old were used. Arena was an open filed made up of acrylic (50 x 50 x 50 cm). Twenty four hours prior to testing, rats were habituated to individual test arenas for 20 min in the absence of any objects. 24 h after the habituation, rats (7-8 animals per group) were administered respective treatments. After the post treatment interval of 30 minutes, rats were subjected to familiarization phase (T1). Rats were placed individually in the open field for 3 min, containing two identical objects (a1 and a2). Choice trial (T2) was carried out after 24 h after the T1 trial. Rats were administered respective treatments. After the post treatment interval of 30 min rats were subjected to Choice trial (T2). Rats were allowed to explore the open field for 3 min in presence of one familiar object (a3) and one novel object (b). Exploration time was noted.

**Novel Object Recognition Task (With 5-$HT_4$R selective antagonist GR-125487)**

On day 1 the Wistar rats (n = 7-8/group) were habituated to the arena for 20 minutes. On day 2 or 3, 30 min. before the trial-1 the animals were administered Vehicle (Water for injection, WFI) or compound **4o**, 3 mg/kg, *p.o*. On day 2 or 3, GR-125487 1 mg/kg, s.c. was administered 40 min before the trial. The Trial-1 is familiarization task in the arena with 2 yellow bottles of same dimensions for a period of 3 minutes. Trial-II is recognition task in the arena with one yellow and one black bottle for a period of 3 minutes. The vehicle treated rats spent almost equal time exploring the novel object as well as the familiar object in the test i.e. they did not remember the familiar object. Rats treated with compound **4o** at 3 mg/kg, p.o. spent significantly more time exploring the novel object than the familiar object in the test phase. Rats treated with GR-125487 at 1mg/kg, *s.c.* spent almost equal time exploring the novel object as well as the familiar object in the test phase. Groups treated with **4o**, 3 mg/kg, *p.o.* & GR 125487, 1 mg/kg, *s.c.* combination spent almost equal time exploring the novel object as well as the familiar object in the test phase. GR-125487 antagonized or blocked the action of compound **4o**.

**Determination of *In Vivo* receptor occupancy (R.O)**

Male Wistar rats (*n* = 4/group) were administered with vehicle or test compounds (0.3, 1, 3, 10 and 30 mg/kg, *p.o*.). After 1 h of treatment with test compound or vehicle, rats were restrained and administered with tracer (SB207145, 1 µg/kg) intravenously. After 30 minutes of tracer administration, rats were sacrificed and brain was separated for isolation of cerebellum (non-specific region) and striatum (specific region). Isolated brain tissues were transferred to pre-labeled vials and stored on dry ice until quantification of tracer (SB207145) using LC-MS/MS.

Percent receptor occupancy was calculated using the concentration of tracer in the (striatum) to the cerebellum (receptor null region).

***In Vivo* Brain Microdialysis**.[40]

Animals were placed in a stereotaxic frame with the incisor bar set at 3.2 mm under the horizontal plane passing through the interaural line. A microdialysis guide cannula (CMA/11, CMA Microdialysis, Stockholm, Sweden) was implanted into the frontal cortex (AP +3.2 mm, ML -3.2 mm, DV –1.5 mm). Coordinates were taken according to the Paxinos and Watson (2004)* with reference points taken from bregma and vertical from the skull. On the day of experiment, pre-equilibrated microdialysis probe (CMA/11, 2 mm, CMA Microdialysis, Stockholm, Sweden) was inserted into the guide cannula directing the dialysis membrane in frontal cortex. The probe was perfused with aCSF at 1.5 µL/min set by a micro-infusion pump (Picoplus, Harvard, MA, USA). After stabilization period of 2 h, sample collection was initiated with a sampling interval of 20 min; five basal samples were collected prior to administration of compound **4o** (3 or 10 mg/kg, *p.o*.) and dialysate samples were collected for an additional period of 4 h. Samples were stored below −70 °C until quantification of acetylcholine by LC-MS/MS..

**Estimation of mice brain cortical sAPPα levels**.[41,42,43]

Male C57BL/6J mice (20 - 30 grams) were randomly divided (n=7/ group) into different treatment groups. Control group of mice were subcutaneously (*s.c*.).administered with sterile water for injection. Mice from treatment groups received a single *s.c*. injection of test compound (dose volume of 10 mL/kg) or prucalopride (10 mg/kg) dissolved in sterile water for injection. Mice were sacrificed by cervical dislocation at 60 minutes or 90 minutes after administration of test compound, or prucalopride, respectively. Brains were quickly isolated and the cortex was

dissected at −20 °C. The cortex was immediately kept on a dry ice and weighed before being stored at −80 °C until quantification of sAPPα using Enzyme-linked immunosorbent assay (ELISA) using commercially available kits from IBL (currently known as Tecan, Männedorf Switzerland). Statistical analyses were performed using the GraphPad Prism (Version 4). Data are Mean ± SD of sAPPα levels expressed as percentage of control values (mice which received water for injection). Values were compared between the different groups by using unpaired test. The significance level was set at *p<0.05; **p<0.01; ***p<0.001.

## ASSOCIATED CONTENT

### Supporting Information

The supporting information is available free of charge on the ACS publication website at DOI:

Experimental procedures, characterization data of final compounds, comparative *in vitro* affinities of 5-$HT_4$R reference standards, data from functional assays, protein binding results, selectivity profile and elemental analysis

Molecular formula strings (CSV)

## AUTHOR INFORMATION

### Corresponding Author

*e-mail, nvsrk@suven.com

ORCID: 0000-0002-2045-0784

### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript and declare no competing financial interest.

**ACKNOWLEDGEMENTS**

The support of scientists from analytical chemistry, in vitro and in vivo Pharmacology, Drug Metabolism and Pharmacokinetics Departments; Moral and financial support from Mr. Venkateswarlu Jasti, CEO, Suven Life Sciences Ltd., Hyderabad is greatly acknowledged.

**ABBREVIATIONS USED**

AD, Alzheimer's Disease; SAR, Structure-Activity Relationships; CNS, Central Nervous System; 5-$HT_4$R, 5-Hydroxytryptamine 4 Receptor; *p.o*., oral dosing; *s.c*., subcutaneous dosing; min, minutes; mm, millimeter; rpm, rounds per minute; cps, counts per second; hERG, human Ether-à-go-go-Related Gene. sAPPα, soluble amyloid precursor protein α; R.O., receptor occupancy; LC-MS/MS, Liquid Chromatography-Mass Spectrometry; HPLC, high performance liquid chromatography; Ach, acetylcholine; $EC_{50}$, concentration giving a 50% response; $ED_{50}$, median effective dose; $E_{max}$, maximal effect; GPCR, G-protein-coupled receptor; HEK, human embryonic kidney; HLM, human liver microsome; RLM, rat liver microsome; SEM, standard error of the mean; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; THP, tetrahydropyran; DCM, dichloromethane; TLC, thin layer chromatography; *n*-BuLi, *n*-butyllithium; IPA, isopropanol, EtOAc, ethylacetate; TBTU, 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate; HATU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate.

## REFERENCES


1. Gerald, C.; Adham, N.; Kao, H. T.; Olsen, M. A.; Laz, T. M.; Schechter, L. E.; Bard, J. A.; Vaysse, P. J.-J. Hartig, P. R.; Branchek, T. A.; Weinshank, R. L. The 5-$HT_4$ receptor: Molecular cloning and pharmacological characterization of two splice variants. *The EMBO Journal*, **1995**, *14*, 2806-2815.

2. Maillet, M.; Robert, S. J.; Lezoualc'h, F. New insights into serotonin 5-$HT_4$ receptors: A novel therapeutic target for Alzheimer's disease? *Curr. Alzheimer Res*. **2004**, *1*, 79-85.

3. Tonini, M.; Fabio, P. Drugs acting on serotonin receptors for the treatment of functional GI disorders. *Dig Dis*., **2006**, *24*, 59-69.

4. Bouras, E. P.; Camilleri, M.; Burton, D. D.; Mckinzie, S. Selective stimulation of colonic transit by the benzofuran 5-$HT_4$ agonist prucalopride, in healthy humans. *Gut*, **1999**, *44*, 682-686.

5. Bockaert, J., Claeysen, S., Compan, V., Dumuis, A.; 5-$HT_4$ receptors, a place in the sun: act two. *Curr. Opin. Pharmacol*. **2011**, *11*, 87-93.

6. Lezoualc'h, F., Robert S. J. The serotonin 5-$HT_4$ receptor and the amyloid precursor protein processing. *Exp. Gerontol*. **2003**, *38*, 159-66.

7. Robert, S. J.; Zugaza, J. L.; Fischmeister, R.; Gardier, A. M.; Lezoualc'h, F. The human serotonin 5-$HT_4$ receptor regulates secretion of non-amyloidogenic precursor protein. *J. Biol. Chem.* **2001**, *276*, 44881-44888.

8. Bockaert, J., Claeysen, S., Compan, V., Dumuis, A. 5-$HT_4$ receptors. *Curr. Drug Targets*, **2004**, *3*, 39-51.

9. Brodney, M. A.; Johnson, D. E.; Sawant-Basak, A.; Coffman, K. J.; Drummond, E. M.; Hudson, E. L.; Fisher, K. E.; Noguchi, H.; Waizumi, N.; McDowell, L. L.; Papanikolaou, A.; Pettersen, B. A.; Schmidt, A. W.; Tseng, E.; Stutzman-Engwall, K.; Rubitski, D. M.; Vanase-Frawley, M. A.; Grimwood, S. Identification of multiple 5-$HT_4$ partial agonist clinical candidates for the treatment of Alzheimer's disease. *J. Med. Chem*. **2012**, *55*, 9240-9254.

10. Ahmad, I.; Nirogi, R. 5-$HT_4$ receptor agonists for the treatment of Alzheimer's disease. *Neurosci. Med*. **2011**, *2*, 87-92.

11. Langlois, M.; Fischmeister, R. 5-$HT_4$ receptor ligands: applications and new prospects. *J. Med. Chem.* **2003**, *46*, 319-344.

12. Modica, M. N.; Pittala, V.; Romeo, G.; Salerno, L.; Siracusa, M. A. Serotonin 5-$HT_3$ and 5-$HT_4$ ligands: An update of medicinal chemistry research in the past few years. *Curr. Med. Chem.* **2010**, *17*, 334-362.

13. Castriconi, F.; Paolino, M.; Giuliani, G.; Anzini, M.; Campiani, G.; Mennuni, L.; Sabatini, C.; Lanza, M.; Caselli, G.; De Rienzo, F.; Menziani, M. C.; Sbraccia, M.; Molinari, P.; Costa, T.; Cappelli, A. Synthesis and structure-activity relationship studies in serotonin 5-$HT_4$ receptor ligands based on a benzo[*de*][2,6]naphthridine scaffold. *Eur. J. Med. Chem*. **2014**, *82*, 36-46.

14. Lead product candidate-PRX-03140 available at: http://library.corporate-ir.net/library/91/917/91717/items/297502/EPIX-FactSheet-Alz-071708a.pdf. Accessed on April 23, 2018.

15. EPIX pharmaceuticals reports compelling clinical results for PRX-03140 in Alzheimer's disease. https://www.businesswire.com/news/home/20071218005206/en/EPIX-Pharmaceuticals-Reports-Compelling-Clinical-Results-PRX-03140. Accessed on April 23, 2018.

16. Marchetti-Gauthier, E.; Roman, F. S.; Dumuis, A.; Bockaert, J.; Soumireu-Mourat, B. BIMU1 increases associative memory in rats by activating 5-$HT_4$ receptors. *Neuropharmacology*, **1997**, *36*, 697-706.

17. Giannoni, P.; Gaven, F.; de Bundel, D.; Baranger, K.; Marchetti-Gauthier, E.; Roman, F. S.; Valjent, E.; Marin, P.; Bockaert, J.; Rivera, S.; Claeysen, S. Early administration of RS 67333, a specific 5-$HT_4$ receptor agonist, prevents amyloidogenesis and behavioral deficits in the 5XFAD mouse model of Alzheimer's disease. *Front Aging Neurosci*., **2013**, *5*, 96.

18. Ramakrishna, N.; Anil, S., Rama Sastry, K.; Abdul Rasheed, M.; Sangram, K. S.; Rajesh kumar, B.; Thrinath, Reddy B.; Venugopalarao, B.; Kumar B.; Veena R.; Ramkumar S.; Vijay B.; Raghava Choudary, P.; Gopinadh, B.; Pradeep J.; Vinod, G.; Venkat J. Discovery and development of 1-[(2-bromophenyl)sulfonyl]-5-methoxy-3-[(4-methyl-1-piperazinyl)methyl]-1H-indole dimesylate monohydrate (SUVN-502): A novel, potent, selective and orally active serotonin 6 (5-$HT_6$) receptor antagonist for potential treatment of Alzheimer's disease. *J. Med. Chem*. **2017**, *60*, 1843-1859.

19. Ramakrishna, N.; Abdul Rasheed, M.; Anil, S., Narsimha, B.; Shankar Reddy, G.; Srinivasa Rao, R.; Laxman, K.; Gopinadh, B.; Nageswara Rao, M.; Vijay, B.; Raghava Chowdary, P.; Pradeep, J.; Ramkumar, S.; Vinod G. Synthesis and SAR of imidazo[1,5-

*a*]pyridine derivatives as 5-$HT_4$ receptor partial agonists for the treatment of cognitive disorders associated with Alzheimer's disease. *Eu. J. Med. Chem*. **2015**, *103*, 289-301.

20. Ramakrishna N.; Anil, S., Vinay Kumar, T.; Laxman, K.; Sangram, K. S.; Rajesh kumar, B.; Abdul Rasheed, M.; Ramkumar S.; Nageshwararao M.; Gopinadh B.; Renny A. Benzamide derivatives and their constrained analogs as histamine $H_3$ receptor antagonists. *Eu. J. Med. Chem*. **2016**, *108*, 655-662.

21. A study to investigate the safety, tolerability and pharmacokinetics of SUVN-D4010 in healthy subjects. Available at https://clinicaltrials.gov/ct2/results?cond=&term=suvn-D4010&cntry=&state=&city=&dist=. Accessed on April 23, 2018.

22. Yang, D.; Soulier, J. L.; Sicsic, S.; Mathé-Allainmat, M.; Brémont, B.; Croci, T.; Cardamone, R.; Aureggi, G.; Langlois, M. New esters of 4-amino-5-chloro-2-methoxybenzoic acid as potent agonists and antagonists for 5-$HT_4$ receptors. *J. Med. Chem*. **1997**, *40*, 608-621.

23. McKinnell, R. M.; Armstrong, S. R.; Beattie, D. T.; Fatheree, P. R.; Long, D. D.; Marquess, D. G.; Shaw, J. P.; Vickery, R. G. Discovery of TD-8954, a clinical stage 5-$HT_4$ receptor agonist with gastrointestinal prokinetic properties. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 4210-4215.

24. Tian, W. Q.; Wang, Y. A. Mechanisms of Staudinger reactions within density functional theory. *J. Org. Chem*. **2004**, *69*, 4299-4308.

25. Nicholas, A. M. Synopsis of some recent tactical application of bioisosteres in drug design. *J. Med. Chem*. **2011**, *54*, 2529.

26. Bostrom, J.; Hogner, A; Llinas, A.; Wellner, E.; Plowright, A. T. Oxadiazoles in medicinal chemistry. *J. Med. Chem*. **2012**, *55*, 1817-1830.

27. Jose, B.; Charles, S. F.; Graham. F. J.; Karen. A. J.; Frank. D. K.; Wesley, D. M.; Gareth, J. S. 5-Hydroxytryptamine (5-$HT_3$) receptor antagonists. 1. Indazole and indolizine-3-carboxylic acid derivatives. *J. Med. Chem*. **1990**, *33*, 1924.

28. Aarti, S. B.; Karen, J.; Coffman, G. S.; Walker, T. F.; Ryder, E. T.; Emily, M.; Carlos, Lee, M.; Vanase, F.; John, W. W.; Michael, A. B.; Tracey, Scott, O. Metabolism of a serotonin-4 receptor partial agonist 4-{4-[4-tetrahydrofuran-3-yloxy)-benzo[d]Isoxazol-3-yloxymethyl]-piperidin-1-ylmethyl}-tetrahydropyran-4-ol (TBPT): Identification of an unusual pharmacologically active cyclized oxazolidine metabolite in human. *J. Pharm. Sci*. **2013**, *102*, 3277-3293.

29. Antunes, M.; Biala, G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn. Process*. **2012**, *13*, 93-110.

30. Nirogi, R.; Abraham, R.; Jayarajan, P.; Medapati, R. B.; Shanmuganathan, D.; Kandikere, V.; Irappanavar, S.; Saralaya, R.; Benade, V.; Bhyrapuneni, G.; Muddana, N. Difference in the norepinephrine levels of experimental and non-experimental rats with age in the object recognition task. *Brain Res*. **2012**, *1453*, 40-45.

31. Letty, S.; Child, R.; Dumuis, A.; Pantaloni, A.; Bockaert, J.; Rondouin, G. 5-$HT_4$ receptors improve social olfactory memory in the rat. *Neuropharmacology*, **1997**, *36*, 681–687.

32. Marchetti-Gauthier, E.; Roman, F. S.; Dumuis, A.; Bockaert, J.; Soumireu-Mourat, B. BIMU1 increases associative memory in rats by activating 5-$HT_4$ receptors. *Neuropharmacology*, **1997**, *36*, 697-706.

33. Nirogi, R.; Kandikere, V.; Bhyrapuneni, G.; Saralaya, R.; Ajjala, D. R.; Aleti, R. R.; Mohammed, A. R. *In vivo* rat striatal 5-$HT_4$ receptor occupancy using non-radiolabelled SB207145. *J. Pharm. Pharmacol*. **2013**, *65*, 704-712.

34. Grimwood, S.; Drummond, E.; Zasadny, K.; Skaddan, M.; Brodney, M.; Coffman, K.; Nicholas, T.; Duvvuri, S.; Raunig, D. L.; Leurent, C.; Rowinski, C.; Park, Y-S.; Ogden, A.; Sawant-Basak, A.; Miller, E. L; Rapp, T.; Wang, Y.; Planeta-Wilson, B.; Ropchan, J.; Carson, R.; Morris, E.; Ding, Y-S.; Katz, E. Translational receptor occupancy for the 5-$HT_4$ partial agonist PF-04995274 in rats, non-human primates and healthy volunteers. *International Conference on Alzheimer's Disease*, July 16-21, **2011**, Poster No. 11818.

35. Johnson, D. E.; Drummond, E.; Grimwood, S.; Sawant-Basak, A.; Miller, E.; Tseng, E.; McDowell, L. L.; Vanase-Frawley, M. A.; Fisher, K. E.; Rubitski, D. M.; Stutzman-Engwall, K. J.; Nelson, R. T.; Horner, W. E.; Gorczyca, R. R.; Hajos, M.; Siok, C. J. The 5-hydroxytryptamine4 receptor agonists, prucalopride and PRX-03140 increase acetylcholine and histamine levels in the rat prefrontal cortex and the power of stimulated hippocampal θ oscillations. *J. Pharmacol. Exp. Ther*. **2012**, *341*, 681-691.

36. Robert, S. J.; Lezoualc'h, F. Distinct functional effects of human 5-$HT_4$ receptor isoforms on beta-amyloid secretion. *Neurodegenerative Diseases*, **2008**, *5*, 163-165.

37. Cachard-Chastel, M.; Lezoualc'h, F.; Dewachter, I.; De- lomenie, C.; Croes, S. Devijver, S.; Langlois, M.; Leuven, F. V.; Sicsic, S.; Gardier, A. M. 5-$HT_4$ receptor agonists

increase sAPPα levels in the cortex and hippocampus of male C57BL/6j mice. *Br. J. Pharmacol.* **2007**, *150*, 883-892.

38. S. Cho, S.; Hu, Y. Activation of 5-$HT_4$ receptors inhibits secretion of β-amyloid peptides and increases neuronal survival. *Experimental Neurology*, **2007**, *203*, 274-278.

39. Curtis R. K.; Najam A. S. Pharmacological evidence for a functional serotonin-2b receptor in a human uterine smooth muscle cell line. *J. Pharmacol. Exp. Ther*. **2006**, *317*, 1254-1261.

40. Mohler, E. G.; Shacham, S.; Noiman, S.; Lezoualc'h, F.; Robert, S.; Gastineau, M.; Rutkowski, J.; Marantz, Y.; Dumuis, A.; Bockaert, J.; Gold, P. E.; Ragozzino, M. E. VRX-03011, a novel 5-$HT_4$ agonist, enhances memory and hippocampal acetylcholine efflux. *Neuropharmacology*, **2007**, *53*, 563-573.

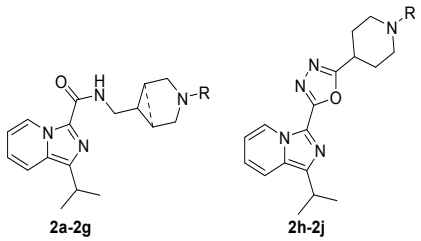
41. Thomas, A. L.; Carol, S. H.; Giovanni, P.; Lisa, A.; Shinji, Y.; Ben, A.; Kalpana, M. M. The γ-Secretase inhibitor *N*-[*N*-(3,5-difluorophenacetyl)-l-alanyl]-*S*-phenylglycine *t*-butyl ester reduces Aβ levels *in vivo* in plasma and cerebrospinal fluid in young (plaque-free) and aged (plaque-bearing) Tg2576 mice. *J. Pharmacol. Exp. Ther*. **2003**, *305*, 864-871.

42. Mohammed, E.; Marc, V.; Theo, M.; Marc, M. Reduction of Aβ levels in the Sprague Dawley rat after oral administration of the functional γ-secretase inhibitor, DAPT: A novel non-transgenic model for Aβ production inhibitors. *Current Pharmaceutical Design,* **2006**, *12*, 671-676.

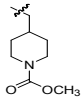
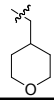
43. Jonathan D.; Best, M. T.; Jay, F. O.; Ian, C.; Michael, R.; Pablo, M.; Christine, P.; Tim, H.; Mark, S. S.; John, R. A. *In Vivo* Characterization of Aβ(40) changes in brain and

cerebrospinal fluid using the novel γ-secretase inhibitor *N*-[cis-4-[(4-chlorophenyl)sulfonyl]-4-(2,5-difluorophenyl)cyclohexyl]-1,1,1-trifluoromethanesulfonamide (MRK-560) in the rat. *J. Pharmacol. Exp. Ther*. **2006**, *317*, 786-790.

**Table 1**. Showing 5-$HT_4R$ compounds **2a-2j** functional activity, *in vitro* metabolism, *in vivo* pharmacokinetics and brain penetration.

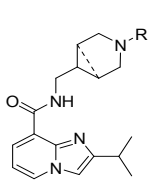


**2a-2g** **2h-2j**

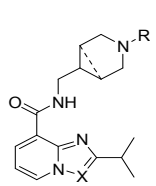
| Compound | Piperidine | R | $EC_{50}$ (nM) /Emax[a] | % Metabolized RLM | % Metabolized HLM | %F | $C_{max}$ ng/mL | AUC ng*hr/mL | CL (mL/min/kg) | $C_b/C_p$ @ 1h |
|---|---|---|---|---|---|---|---|---|---|---|
| **PRX-03140** | N.A | N.A | 58/48% | 36.9 ± 8.1 | 22.1 ± 1.3 | 2 ± 0 | 17 ± 5 | 22 ± 0 | 62 ± 10 | 1.5 ± 0.4 |
| **PF-04995274** | N.A | N.A | 1.5/18% | 87.3 ± 16.1 | 71.9 ±13.9 | 5.4 ± 0.3 | 85 ± 11 | 94 ± 5 | 29 ± 5 | 0.7 ± 0.2 |
| **Prucalopride** | N.A | N.A | 5.2/80% | 36.9 ± 8.1 | 22.1 ± 1.3 | 84 ± 12 | 285 ± 64 | 828 ± 129 | 113 ± 12 | 0.5 ± 0.2 |
| **2a** | B1 |  | 48/82% | 28.2 ± 1.6 | 27.3 ± 1.1 | ND | ND | ND | ND | ND |
| **2b** | B1 |  | 65/17% | 19.7 ± 0.7 | 13.2 ± 0.5 | 7 ± 1 | 34 ± 3 | 48 ± 8 | 77 ± 7 | 1.8 ± 0.3 |
| **2c** | B1 |  | 4.9/9% | 59.2± 2.7 | 65.6 ± 1.4 | 7 ± 3 | 55 ± 42 | 52 ± 26 | 67 ± 5 | 1.4 ± 0.6 |
| **2d** | B1 |  | 8.8/12% | 61.8 ± 0.6 | 39.8 ± 2.9 | 25 ± 13 | 171 ± 51 | 161 ± 85 | 77 ± 3 | 2.6 ± 1.1 |
| **2e** | B1 |  | 42/21% | 43.2 ± 0.9 | 34.1 ± 1.9 | ND | ND | ND | ND | ND |
| **2f** | B2 |  | 13.6/32% | 11.3 ± 0.7 | 08.2 ± 1.5 | 23 ± 1 | 92 ± 18 | 151 ± 6 | 74 ± 5 | 2 ± 0 |
| **2g** | B2 |  | 10.4/41% | 0.0 | 24.4 ± 2.7 | 15 ± 0.1 | 60 ± 9 | 121 ± 1 | 62 ± 1 | 4 ± 1 |
| **2h** | B1 |  | 758/81% | 76.6 ± 4.5 | 11.5 ± 2.9 | ND | ND | ND | ND | ND |
| **2i** | B1 |  | 796/82% | 48.0 ± 6.7 | 17.3 ± 2.8 | ND | ND | ND | ND | ND |
| **2j** | B1 |  | 484/86% | 38.4 ± 9.2 | 18.2 ± 7.1 | ND | ND | ND | ND | ND |

[a]Functional activity determined in CHO cells stably expressing the human $5HT_{4E}$ isoform and a reporter gene was used. $EC_{50}$ values were expressed as the geometric mean of two independent experiments performed in triplicates; RLM- Rat Liver Microsomes; HLM-Human Liver Microsomes; CL - Intravenous Clearance; $C_{max}$- Maximum Systemic Concentration; $C_b$-Concentration in brain; $C_p$-Concentration in plasma; AUC- Area under the Curve; $C_b/C_p$ - Brain to plasma concentration ratio; %F - Oral bioavailability; B1- *N*-substituted piperidine ring; B2-*N*-substituted 3-aza-bicyclo[3.1.0]hexane ring, N.A, Not applicable, The reported *in vitro* potency of PRX-03140 (17-52 nM/30-61%), PF-04995274 (0.26 nM/7%) and prucalopride (26 nM/52%) are comparable with in-house data (See supporting information).

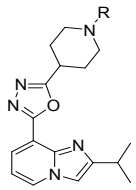
**Table 2**. Showing of 5-$HT_4R$ compounds **3a- 3s** functional activity, *in vitro* metabolism, *in vivo* pharmacokinetics and brain penetration.



**3a-3m**



**3n, 3o, X = CCl**
**3p, 3q, X = N**



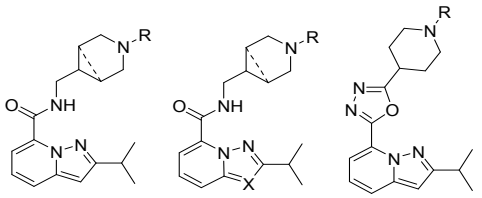
**3r, 3s**

| Compound | Cyclic piperidine | R | $EC_{50}$ (nM) /Emax[a] | % Metabolized RLM | % Metabolized HLM | %F | $C_{max}$ ng/mL | AUC ng*hr/mL | CL mL/min/kg | $C_b/C_p$ @ 1h |
|---|---|---|---|---|---|---|---|---|---|---|
| **3a** | B1 |  | 9.0/57 | 97.5 ± 0.9 | 14.0 ± 3.4 | ND | ND | ND | ND | ND |
| **3b** | B1 |  | 1.5/49 | 78.36 ± 2.3 | 25.5 ± 6.8 | 1.3 ± 0.3 | 15 ± 2 | 7 ± 2 | 91 ± 3 | 1.0 ± 0.4 |
| **3c** | B1 |  | 0.6/51% | 74.9 ± 2.1 | 79.3 ± 0.5 | 4 ± 1 | 22 ± 13 | 21 ± 5 | 104 ± 2 | 6.2 ± 2.4 |
| **3d** | B1 |  | 0.6/42% | 61.3 ± 3.3 | 56.1 ± 2.7 | 1.1 ± 1.2 | 10 ± 10 | 8 ± 9 | 67 ± 5 | 0.6 ± 0.98 |
| **3e** | B1 |  | 8.4/10% | ND | ND | ND | ND | ND | ND | ND |
| **3f** | B1 |  | 0.4/38 | ND | ND | ND | ND | ND | ND | ND |
| **3g** | B1 |  | 0.7/35 | ND | ND | ND | ND | ND | ND | ND |
| **3h** | B1 |  | 0.3/34 | ND | ND | ND | ND | ND | ND | ND |
| **3i** | B2 |  | 34.2/52 | ND | ND | ND | ND | ND | ND | ND |
| **3j** | B2 |  | 13.8/44% | 56.9 ± 9.8 | 11.6 ± 3.2 | 7.0 ± 1.0 | 67 ± 9 | 79 ± 10 | 46 ± 7 | 0.84 ± 1.4 |
| **3k** | B2 |  | 114/47 | ND | ND | ND | ND | ND | ND | ND |
| **3l** | B2 |  | 36/19% | ND | ND | ND | ND | ND | ND | ND |
| **3m** | B2 |  | 17/24 | 91.3 ± 4.5 | 46.3 ± 8.5 | ND | ND | ND | ND | ND |
| **3n** | B1 |  | 1090/2% | 29.2 ± 5.0 | 13.3 ± 1.5 | ND | ND | ND | ND | ND |
| **3o** | B2 |  | >10000 | 19.9 ± 2.9 | 12.6 ± 2.7 | ND | ND | ND | ND | ND |
| **3p** | B1 |  | 1941/3 | 23.4 ± 3.0 | 2.7 ± 0.5 | ND | ND | ND | ND | ND |
| **3q** | B1 |  | 79/6% | 8.9 ± 1.5 | 16.1 ± 1.5 | ND | ND | ND | ND | ND |
| **3r** | B1 |  | 687/64% | 23.1 ± 3.5 | 13.2 ± 1.3 | ND | ND | ND | ND | ND |
| **3s** | B1 |  | 598/16% | 19.1 ± 2.9 | 9.5 ± 0.9 | ND | ND | ND | ND | ND |

[a]Functional activity determined in CHO cells stably expressing the human $5HT_{4E}$ isoform and a reporter gene was used. $EC_{50}$ values were expressed as the geometric mean of two independent

experiments performed in triplicates; RLM- Rat Liver Microsomes; HLM-Human Liver Microsomes; CL - Intravenous Clearance; $C_{max}$- Maximum Systemic Concentration; $C_b$-Concentration in brain; $C_p$-Concentration in plasma; AUC- Area under the Curve; $C_b/C_p$ - Brain to plasma concentration ratio; %F - Oral bioavailability; B1- *N*-substituted piperidine ring; B2- *N*-substituted 3-aza-bicyclo[3.1.0]hexane ring

**Table 3**. Showing 5-$HT_4$R compounds **4a- 4o** functional activity, *in vitro* metabolism, *in vivo* pharmacokinetics and brain penetration.



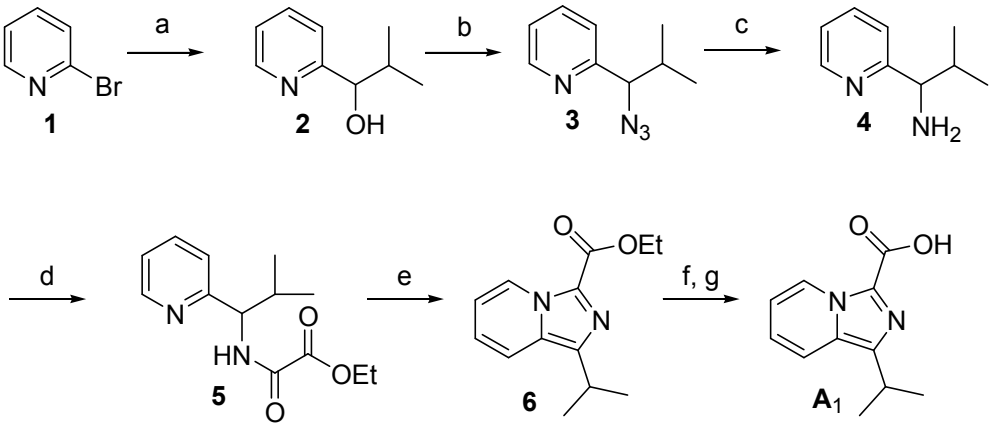
**4a-4f** **4g-4i, X = CCl** **4j-4l, X = N** **4m-4o**

| Compound | Cyclic Piperidine | R | $EC_{50}$ (nM) /Emax[a] | % Metabolized RLM | % Metabolized HLM | %F | $C_{max}$ ng/mL | AUC ng*hr/mL | CL mL/min/kg | $C_b/C_p$ @ 1h |
|---|---|---|---|---|---|---|---|---|---|---|
| **4a** | B1 |  | 0.3/26% | 84.0 ± 0.5 | 44.9 ± 1.3 | ND | ND | ND | ND | ND |
| **4b** | B1 |  | 0.1/24% | 79.2 ± 0.4 | 53.8 ± 2.1 | 6 ± 1.7 | 56 ± 47 | 34 ± 10 | 86 ± 7 | 2.2 ± 3.8 |
| **4c** | B1 |  | 98/16% | 92.2 ± 0.1 | 19.6 ± 2.2 | 2.6 ± 1.8 | 14 ± 8.0 | 16 ± 11 | 81 ± 13 | 0.0 ± 0.0 |
| **4d** | B2 |  | 8.7/29% | 91.9 ± 0.1 | 68.8 ± 2.6 | ND | ND | ND | ND | ND |
| **4e** | B2 |  | 7.3/15% | 95.2 ± 1.7 | 82.5 ± 0.8 | ND | ND | ND | ND | ND |
| **4f** | B2 |  | 8.4/30% | 98.4 ± 0.0 | 96.2 ± 0.1 | ND | ND | ND | ND | ND |
| **4g** | B1 |  | >10000 | 79.1 ± 19.3 | 76.8 ± 20.2 | ND | ND | ND | ND | ND |
| **4h** | B2 |  | >10000 | 64.5 ± 11.9 | 49.9 ± 13.6 | ND | ND | ND | ND | ND |
| **4i** | B2 |  | 291/32% | 62.0 ± 1.0 | 67.9 ± 0.8 | ND | ND | ND | ND | ND |
| **4j** | B1 |  | >10000 | ND | ND | ND | ND | ND | ND | ND |
| **4k** | B1 |  | 1941/3% | 23.4 ± 4.2 | 2.7 ± 0.6 | ND | ND | ND | ND | ND |
| **4l** | B1 |  | >10000 | 16.1 ± 1.2 | 8.9 ± 1.2 | ND | ND | ND | ND | ND |
| **4m** | B1 |  | 54/85% | 25.2 ± 3.2 | 4.8 ± 3.4 | 127 ± 85 | 1391 ± 884 | 2533 ± 1705 | 25 ± 3 | 0.1 ± 0.0 |
| **4n** | B1 |  | 62/24% | 34.3 ± 4.8 | 18.7 ± 1.7 | 28 ± 5 | 217 ± 56 | 247 ± 47 | 56 ± 4 | 2.8 ± 0.2 |
| **4o** | B1 |  | 21/48% | 37.4 ± 0.7 | 29.9 ± 3.0 | 124 ± 34 | 948 ± 199 | 1753 ± 486 | 35 ± 3 | 0.8 ± 0.1 |

[a] Functional activity determined in CHO cells stably expressing the human $5HT_{4E}$ isoform and a reporter gene was used. $EC_{50}$ values were expressed as the geometric mean of two independent

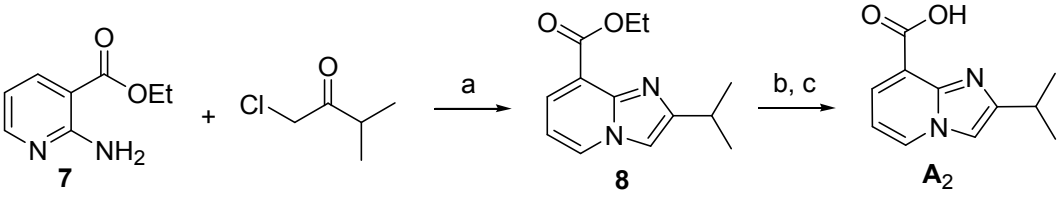
experiments performed in triplicates; RLM- Rat Liver Microsomes; HLM-Human Liver Microsomes; CL - Intravenous Clearance; Cmax- Maximum Systemic Concentration; $C_b$- Concentration in brain; $C_p$ - Concentration in plasma; AUC- Area under the Curve; $C_b/C_p$ - Brain to plasma concentration ratio; %F - Oral bioavailability; B1- *N*-substituted piperidine ring; B2- *N*-substituted 3-aza-bicyclo[3.1.0]hexane ring.

**Scheme 1**. Synthesis of heteroaromatic acid $\mathbf{A_1}$



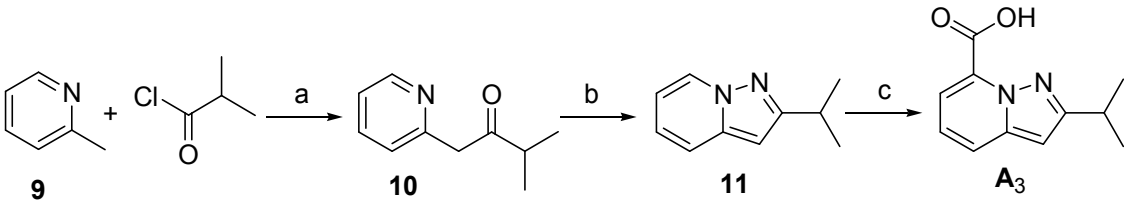

Reagents: a) *n*-BuLi, isobutyraldehyde, THF, –78 °C, 1 h, 56%; b) i. MsCl, $Et_3N$, DCM, 0 °C, 90%; ii. $NaN_3$, $K_2CO_3$, DMF, RT 12 h, 68%; c). $Ph_3P$, THF, $H_2O$, RT, 86%; d) Ethyl chlorooxoacetate, $Et_3N$, DCM, 0 °C 1 h. 90%; e) $POCl_3$, DCE, reflux, 16 h. 27%; f) NaOH, EtOH, $H_2O$, 95 °C, 75%; g) Conc. HCl

**Scheme 2**. Synthesis of heteroaromatic acid $\mathbf{A_2}$



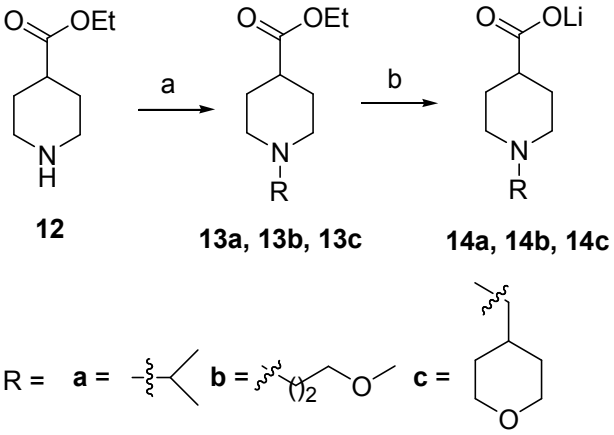

Reagents: a) 1-Chloro-3-methylbutan-2-one, EtOH, 90° C, 16 h. 32%; b) NaOH, THF, $H_2O$, 95 °C, 90%; c) 1N HCl

**Scheme 3**. Synthesis of heteroaromatic acid $\mathbf{A_3}$



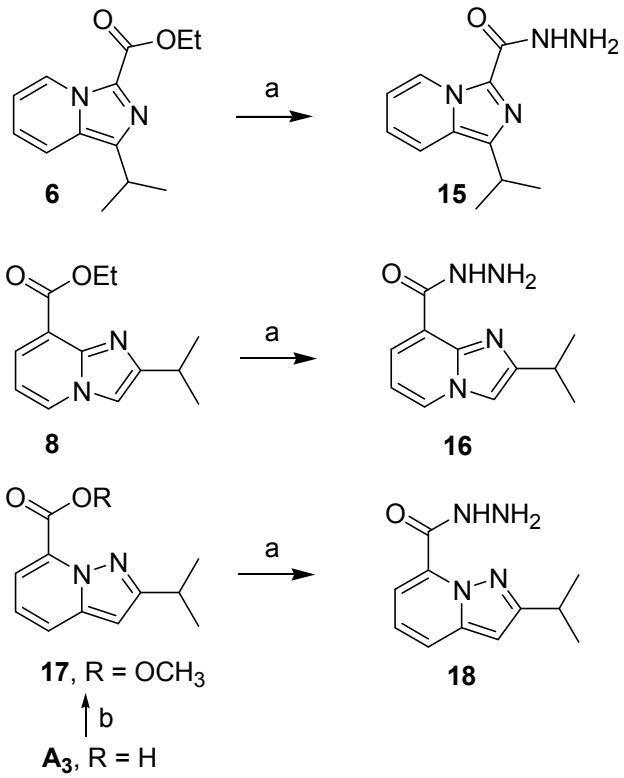

Reagents: a) *n*-BuLi, THF, isobutyryl chloride, −78 °C, 50% b) *O*-Mesitylenesulfonylhydroxylamine, $CHCl_3$, r.t., 60% c) *n*-BuLi, dry ice ($CO_2$ source), −78 °C, 1N HCl, 50%

**Scheme 4**: Synthesis of *N*-substituted lithium isonipecotates



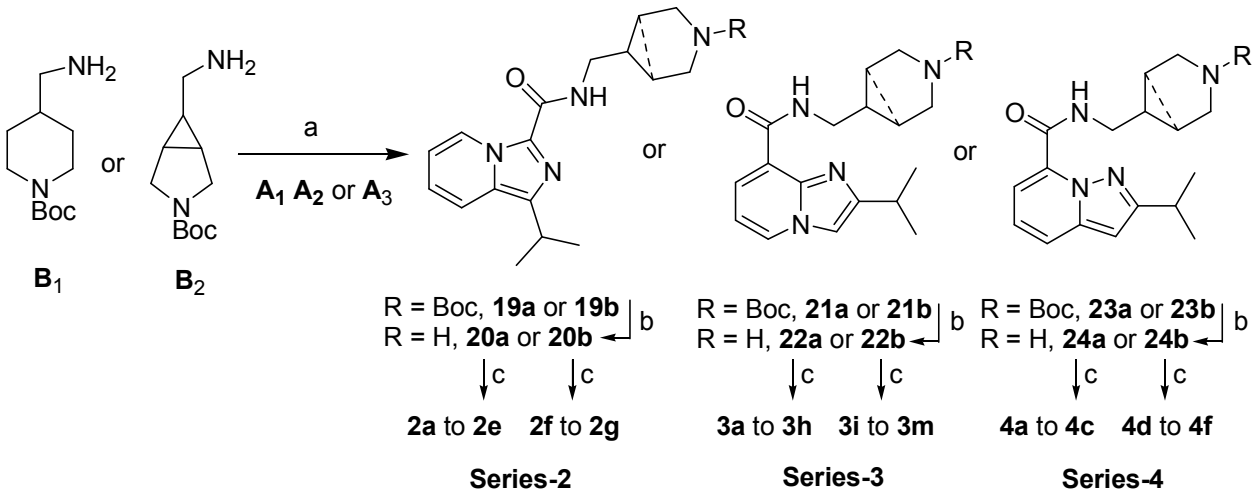

Reagents: a) $Cs_2CO_3$, R-X, AcN, r.t. or R-CHO, $NaBH(OAc)_3$, DCM, 0 °C-r.t.; b) LiOH, THF, 0 °C-r.t.

**Scheme 5**: Synthesis of heteroaromatic hydrazides



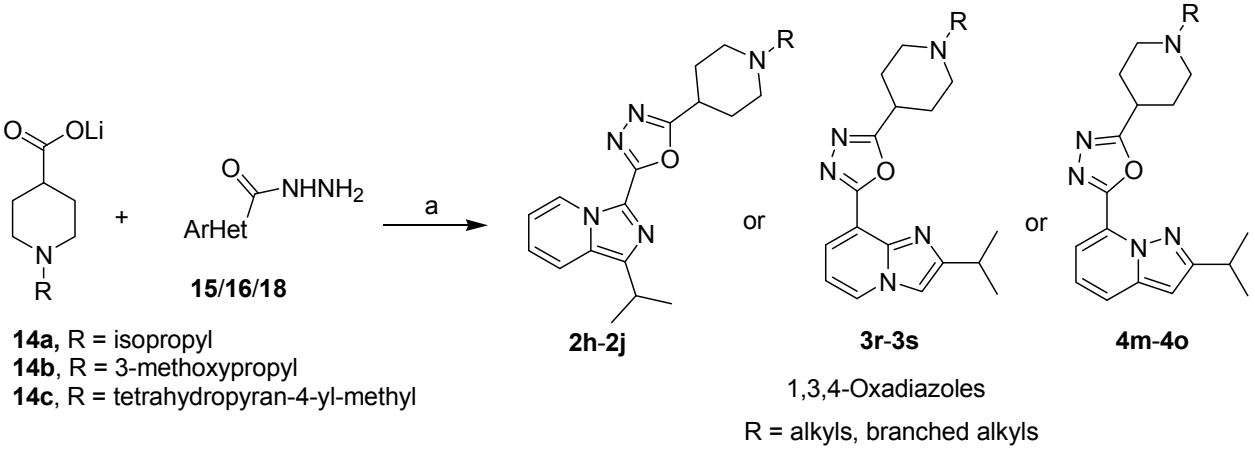

Reagents: a) Hydrazine hydrate, EtOH, reflux; b) $SOCl_2$, MeOH, 0 °C-r.t

**Scheme 6**. Synthesis of heteroaromatic carboxamide derivatives




Reagents: a) TBTU, $EtiPr_2N$, DCM, 50% b) IPA/HCl, 0 °C-r.t., 16 h c) $K_2CO_3$, R-X, AcN, r.t. or R-CHO, $NaBH(OAc)_3$, DCM, 0 °C-r.t.

**Scheme 7**: Synthesis of 1,3,4-oxadiazoles




Reagents: a) $POCl_3$, 120 °C, 4-10 h.

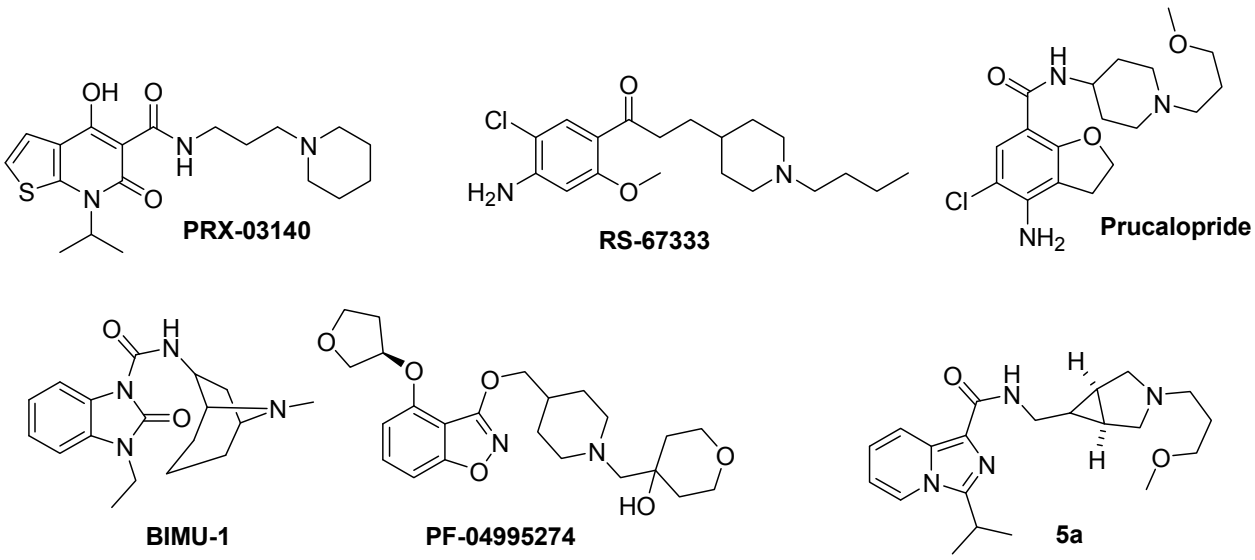


**Figure 1.** Reported 5-HT$_4$R ligands

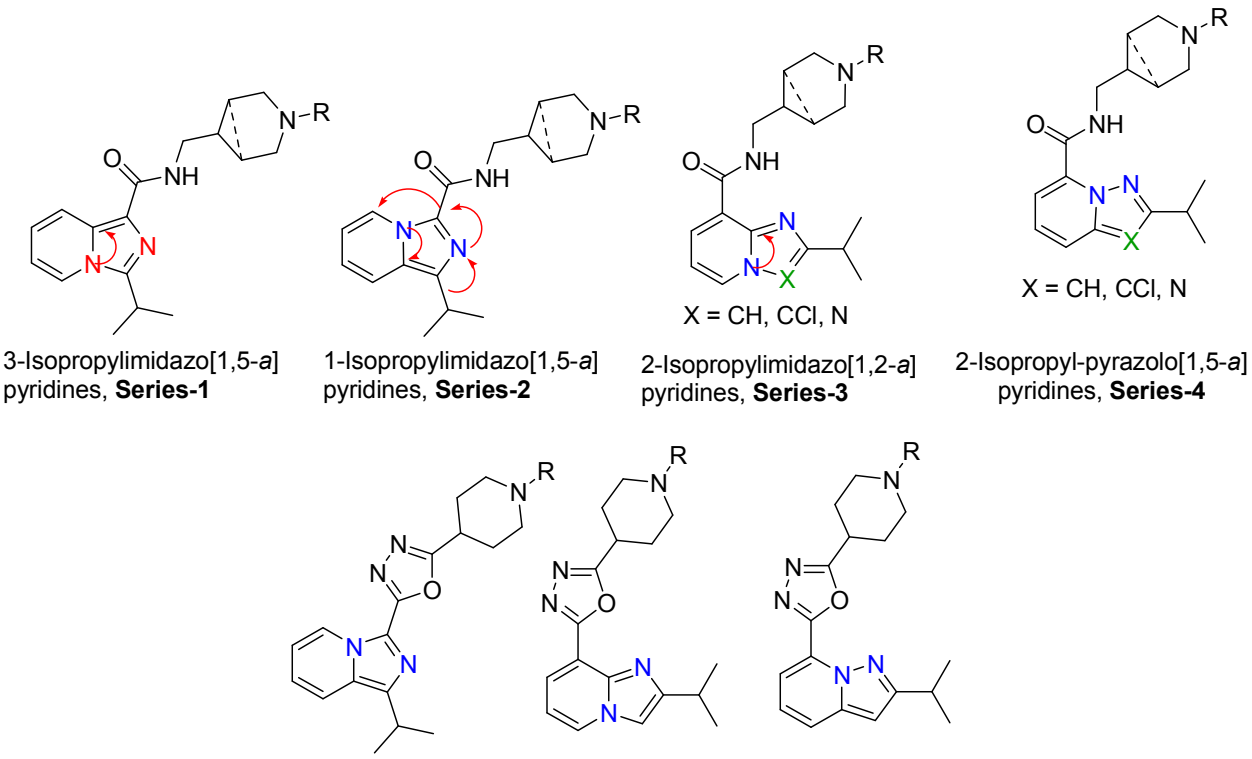
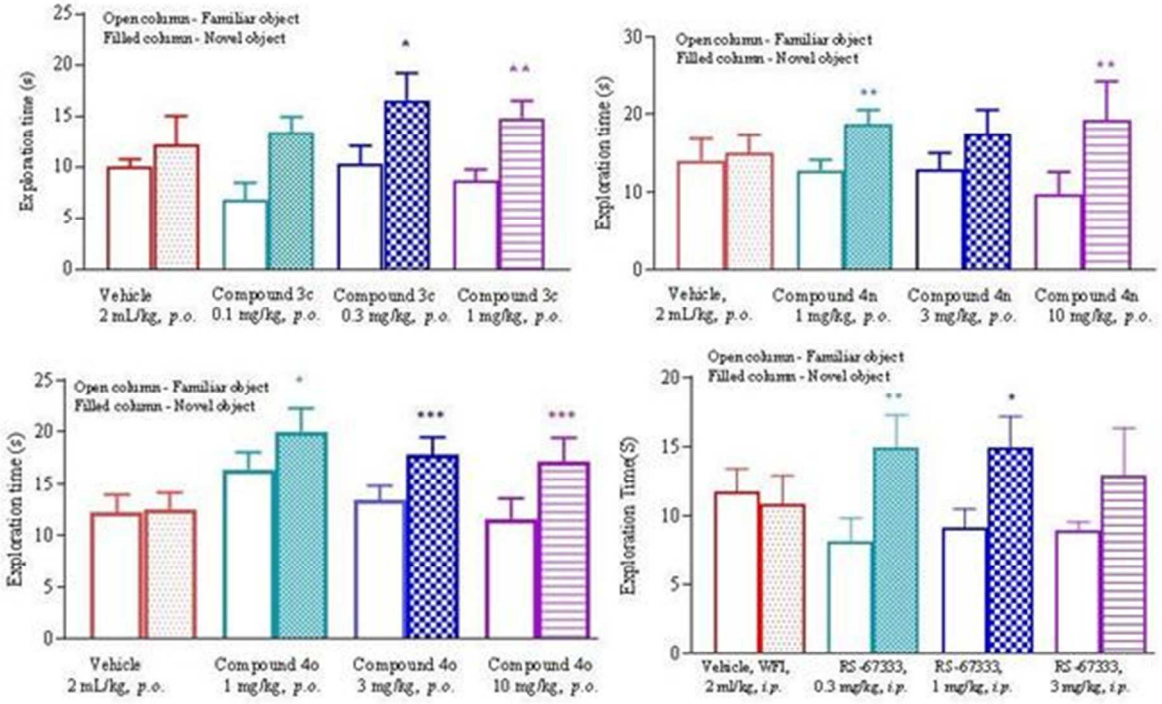
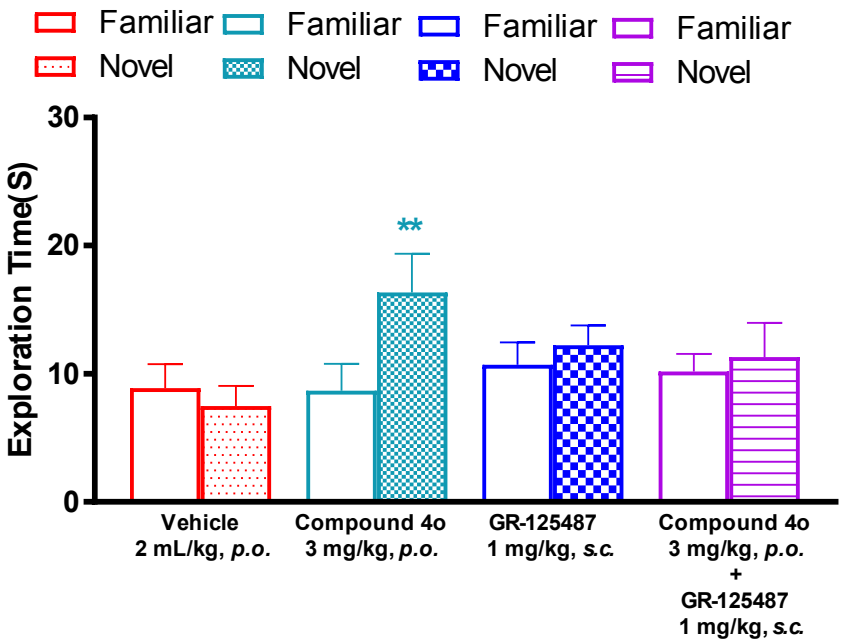



**Figure 2.** Genesis of Ligands



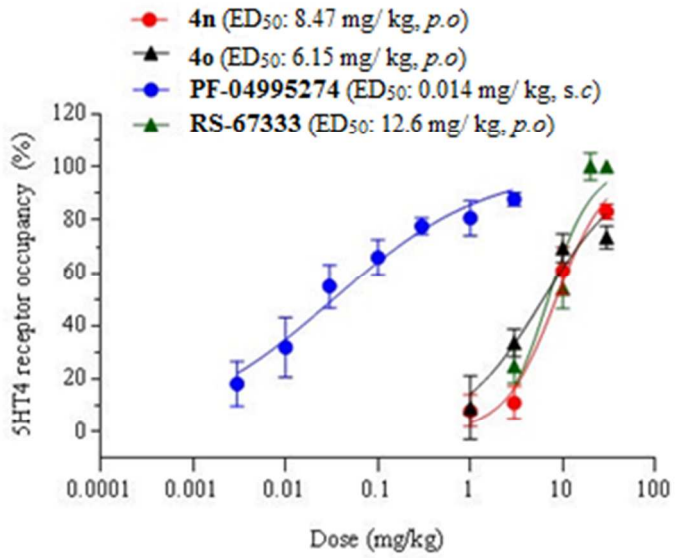

Data represents (Mean ± SEM) of exploration time compared to vehicle (n=7-8/group). *p<0.05, **p<0.01, ***p<0.001 Vs Vehicle (Students paired t-test). Two independent studies were performed

**Figure 3.** Attenuation of long-term memory deficits in NORT by compounds **3c**, **4n**, and **4o**



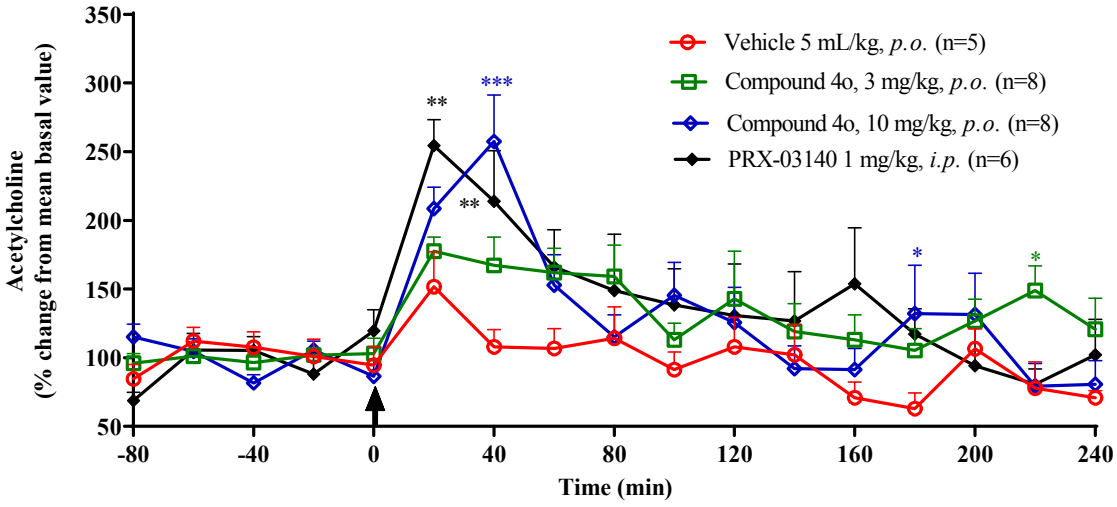
Data represents (Mean ± SEM) of exploration time compared to vehicle (n=7-8/group). **p<0.01 Vs Vehicle (Students paired t-test). Two independent studies were performed

**Figure 4.** Attenuation of long-term memory deficits in NORT by compound **4o** and blockade of its activity by selective 5-HT$_4$R antagonist GR-125487



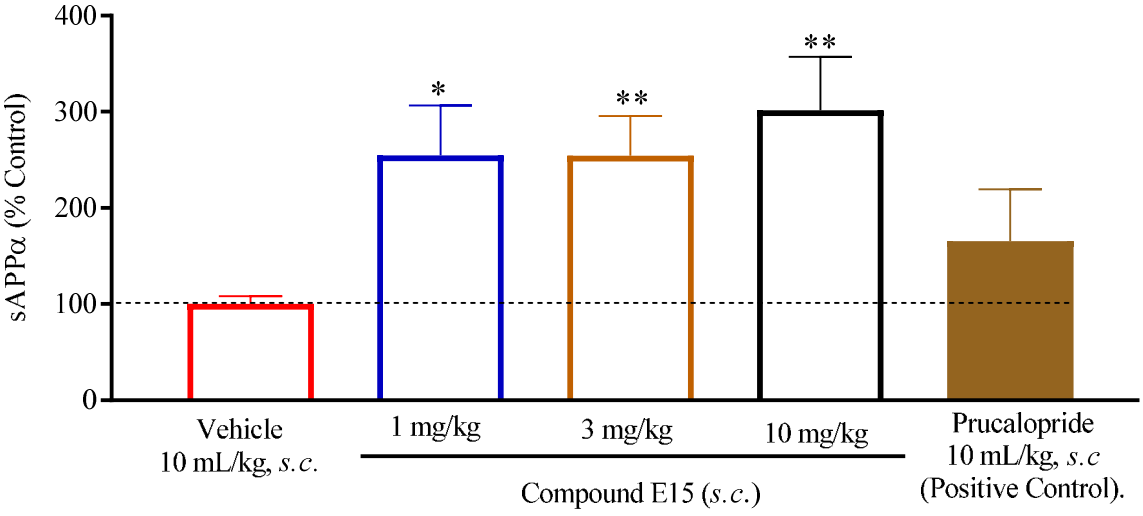

Data shown are the mean ± SEM (n= 4 animals).

**Figure 5.** 5-HT$_4$R occupancy of compounds **4n** and **4o**




Data represents mean ± SEM of percent change in acetylcholine levels. Arrow indicates the point of treatment (n=5-8/group). *p<0.05, ** p<0.01, *** p<0.001 Vs Vehicle (Bonferroni post test).

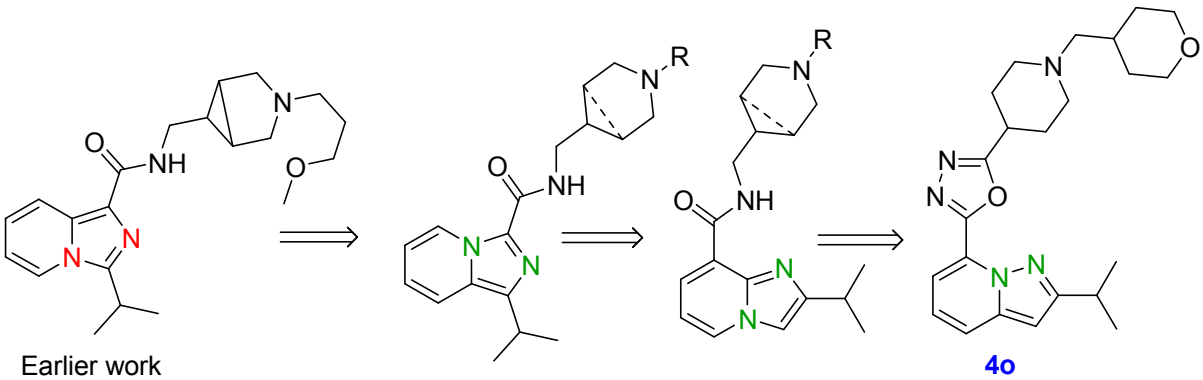
**Figure 6.** Increase in acetylcholine release in the frontal cortex by compound **4o**




Data represents mean ± SEM of % increase relative to vehicle (n=7-8/group). *p<0.05, ** p<0.01 Vs Vehicle (Dunnett's post test).

**Figure 7.** Dose dependent increases in sAPPα levels after subcutaneous (*s.c*) treatment with compound **4o**

## Table of Contents Graphic (TOC)



Earlier work
5-$HT_4$R partial agonist **5a**
$EC_{50}$ = 22 nM
F = 26%
$C_b/C_p$ = 0.3

**4o**

Modified structures as 5-$HT_4$R partial agonists

**4o,** $EC_{50}$ = 21 nM
F = 100%
$C_b/C_p$ = 0.8
Active in NORT, microdialysis and sAPP models