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Article

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Donepezil-based Central Acetylcholinesterase Inhibitors by means of a "Bio-oxidizable" Prodrug Strategy: Design, Synthesis and in vitro Biological Evaluation.

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ABSTRACT

With the aim of reducing side effects of acetylcholinesterase inhibitors (AChEIs) during symptomatic treatment of Alzheimer's disease, we report herein a new class of donepezil-based "bio-oxidizable" prodrugs 1 designed to be converted into dual binding site AChEIs 2. While most of indanone-derived N-benzylpyridinium salts 2 revealed to be highly potent dual binding site hAChEIs (IC50 up to 3 nM), outperforming the standard drug donepezil (IC50 = 11 nM), most of the corresponding 1,4-dihydropyridines 1 were found to be inactive. Promisingly, whereas the selected prodrug 1r showed good permeability in the PAMPA-BBB model and high in vitro antioxidant activity, its conversion to AChEI 2r could be easily achieved under mild conditions when incubated in various oxidizing media. Lastly, both compounds 1r and 2r did not show genotoxicity in vitro and displayed high LD50 values in mice, making this prodrug 1r/drug 2r couple a good candidate for further in vivo biological experiments.

INTRODUCTION

Alzheimer's disease (AD), the most common type of dementia affecting elderly people, is a complex irreversible neurological affection clinically characterized by a progressive loss of cognitive abilities associated with the death of central neuronal cells. Given the ageing of the population over the past decades, AD is becoming one of the main public health issues we have to face in developed countries. While one in nine people age 65 years and older is concerned, approximately one-third of people over 85 years of age are affected by this neurodegenerative disorder.[1] Although the origin of AD is still controversial, amyloid plaques and neurofibrillary tangles are pathological hallmarks, which are held accountable for the neuronal cell death observed in neurocortex and hippocampus regions during post-mortem brain examinations and

for the devastating clinical effects of AD.[2] Mounting evidence indicates that oxidative stress is an important factor contributing to the initiation and progression of AD.[3-6]

Another hallmark of AD is the dysfunction and loss of the basal forebrain cholinergic system, thought to be at the roots of cognitive impairments and deficits in memory. Consequently, improvement of the central cholinergic transmission emerged as a promising approach for the symptomatic treatment of AD. Among the different strategies explored to boost acetylcholine levels in brain regions involved in memory, acetylcholinesterase inhibitors (AChEIs) have been extensively investigated providing the sole palliative treatment of mild to moderate AD approved by the FDA. To date, three AChEIs are launched on the market; namely galantamine, rivastigmine and donepezil. Although these AChEIs show a marked preferential central cholinergic activity, severe peripheral cholinergic side effects restrict their therapeutic potential and lead in the most cases to treatment discontinuation in advanced stages of AD.[7,8] While the development of preventive and curative treatments of AD remains the ultimate goal, the search of central selective AChEIs devoid of adverse effects is still a challenging research topic in the years to come to improve cholinergic treatment of AD.

All these three marketed AChEIs possess a basic nitrogen which is protonated at physiological pH. The resulting temporary positive charge interacts within the active site of the enzyme and contributes significantly to the overall affinity of AChE for these ligands. This acid-base equilibrium can also be held responsible for both peripheral and central cholinergic activity of these AChEIs by allowing their neutral form to cross the blood-brain barrier (BBB).

With the aim to relieve side effects arising from peripheral cholinergic activation, we previously reported on the development of “bio-oxidizable” prodrugs derived from cyclic rivastigmine analogues.[9] This first-generation “bio-oxidizable” prodrugs of AChEIs relies on the

masking of the positive charge that is intended to bind with W86 and to guide the carbamate moiety into a proper position so that carbamylation of Ser200 can occur to promote the pseudo-irreversible inhibition of AChE. A first set of in vivo and ex vivo experiments in mice provided proof-of-concept for central redox-activation of the prodrug **A** (IC_{50} > 1 mM), brain delivery of the drug **B** (IC_{50}=20 nM) and central cholinergic activation. Although peripheral redox-activation of prodrug **A** cannot be ruled out, only limited peripheral cholinergic activation was observed probably because of the permanent positive charge in drug **B** that favors its rapid elimination from the circulation of mice.

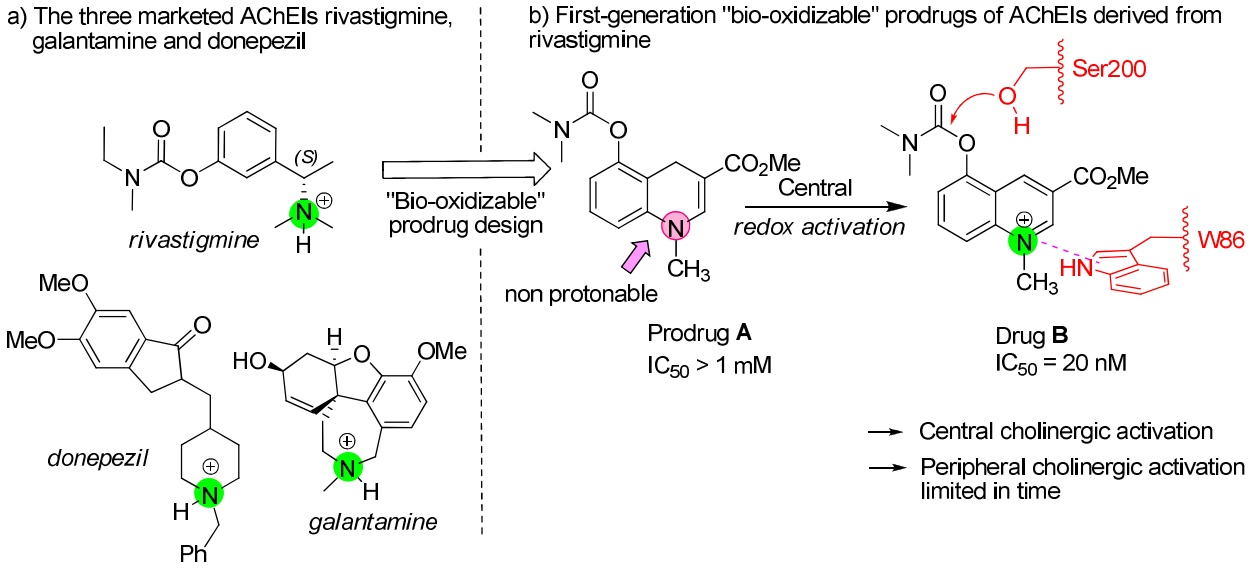


Figure 1. a) The three marketed AChEis rivastigmine, galantamine and donepezil b) First-generation "bio-oxidizable" prodrugs of AChEIs derived from rivastigmine.

More recently, some experimental evidence suggest that central AChE would also play non-cholinergic functions in the development of AD. Indeed, its presence in senile plaques give strong support that central AChE is involved in the aggregation and deposition of β-amyloid peptide (Aβ).[10] More specifically, it has been suggested that the peripheral anionic site (PAS) of

AChE would catalyze β-amyloid peptide (Aβ) aggregation.[10-12] This finding has triggered a renewed interest in the development of new dual AChEIs able to interact with both the catalytic anionic site (CAS) and PAS of AChE.[13-21] Such dual AChEIs are expected not only to alleviate the symptoms but also to slow down the progression of AD.[22-26] Among the three marketed AChEIs, only donepezil displays a dual-binding mode of action (Figure 2a).

In the search of novel central selective AChEIs exhibiting a dual-binding mode of action, we report herein a donepezil-based "bio-oxidizable" prodrug strategy (Figure 2b). This study includes (**1**) the rational design of this second-generation "bio-oxidizable" prodrugs of AChEIs; (**2**) the synthesis of indanone-derived *N*-benzylpyridinium salts **2** as potential new AChEIs and (**3**) their in vitro biological evaluation [AChE and butyrylcholinesterase (BChE) inhibitory activity, propidium iodide displacement, anti-amyloid aggregation activity]; (**4**) a preliminary in vitro evaluation of selected "bio-oxidizable" prodrug **1** /drug **2** couples (*h*AChE inhibitory activity, kinetic studies, PAMPA-BBB permeability, initial assessment of the oxidative activation step of the prodrug **1r** in various media, antioxidant properties of prodrug **1r**, genotoxicity, human neuroblastoma cell viability, docking studies) as well as an evaluation of their acute toxicity in mice.

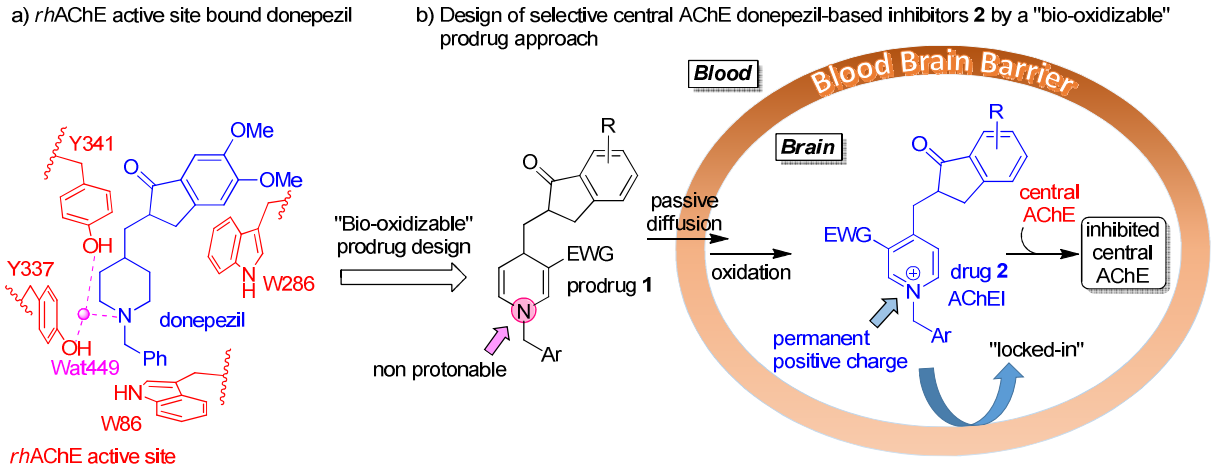


Figure 2. a) Dual-binding mode of donepezil with r*h*AChE active site b) Design of selective central AChE-donepezil-based inhibitors **2** by means of a bio-oxidizable prodrug strategy.

RESULTS AND DISCUSSION

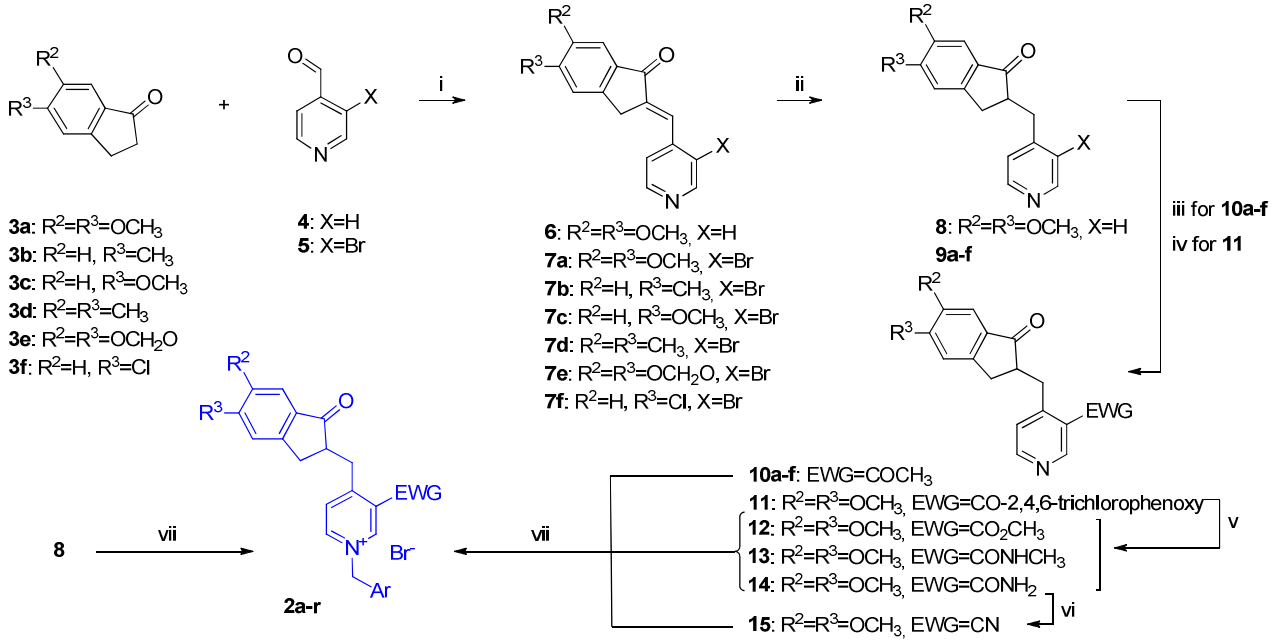
Rational design of a donepezil-based "bio-oxidizable" prodrug system. All the three marketed AChEIs possess a basic nitrogen which is protonated at physiological pH. This resulting temporary positive charge interacts within the active site of the enzyme and contributes significantly to the overall affinity of AChE for these ligands. This acid-base equilibrium can also be held responsible for both peripheral and central cholinergic activity of these AChEIs by allowing their neutral form to cross the blood-brain barrier (BBB). As far as donepezil is concerned, X-ray crystallography and docking studies established that donepezil has a dual-binding mode of action. While the benzyl piperidine residue interacts with the CAS (stacking against W86 in r*h*AChE) and the indanone moiety binds to the PAS (stacking with W286 in r*h*AChE), the protonated piperidine nitrogen makes additional cation-π interactions with aromatic residues at the active site gorge (water-mediated hydrogen bond between piperidine nitrogen and Y341 and Y377 of r*h*AChE).[27,28] The rational design of this prodrug approach is based on the masking of the positive charge at the nitrogen, by replacing the piperidine moiety of

donepezil by a 1,4-dihydropyridine ring. Accordingly, the 1,4-dihydropyridine nitrogen of the prodrug **1**, not basic enough to be protonated at physiological pH, is expected to lead to a significant decrease in AChE inhibitory activity, while being able to cross the BBB as a result of their good lipophilicity. Once in the brain, a redox-activation step mediated by oxidoreductases would convert the 1,4-dihydropyridine prodrug **1** into the corresponding pyridinium drug **2.** The presence of a permanent positive charge within the pyridinium drug **2** is expected not only to restore AChE inhibitory activity but also to entail a "locked-in" effect in the brain, thus preventing peripheral cholinergic adverse effects, while enabling prolonged duration of AChEIs action in brain tissue. At this stage, it is worth noting that an electron-withdrawing group (EWG) at C-3 position of the pyridine moiety constitutes a key element in the design of the prodrug. It is intended to tune the redox properties of the 1,4-dihydropyridine **1**/pyridinium **2** couple so that to provide a prodrug stable enough at the periphery, and which once in the brain can be easily oxidized to exert a central AChE inhibitory activity. Skillful structural variations at the EWG should help to strike a good balance between stability and redox-activation ability of the prodrug. Although 1,4-dihydropyridine-type compounds have been extensively exploited by N. Bodor and L. Prokai as redox targetor to transport and release neuroactive drugs into the brain,[29-33] their use in the design of "bio-oxidizable" prodrug strategies has scarcely been explored.[34-38] Last but not least, the pertinence of this approach was also supported by a survey of the literature which revealed that several *N*-benzyl pyridinium derivatives closely related to the target compounds **2** have shown to exhibit appealing AChE inhibitory activities.[39-46]

Chemistry. In order to conduct a preliminary SAR analysis of this new class of *N*-benzyl pyridinium quaternary donepezil analogues, a set of target compounds **2** were prepared by varying the nature of the withdrawing group (EWG) at C-3 position of the pyridine ring, as well

as by using differently substituted indanones and benzyl groups (Scheme 1). Thus, indanones **3a-f** were reacted with 4-pyridinecarboxaldehydes **4,5** in the presence of PTSA under Dean-Stark conditions to afford the corresponding α,β-unsaturated indanones **6,7a-f** in moderate to good yields (26-79%). Standard catalytic hydrogenation of **6,7a-f** was performed in the presence of platinum on carbon to afford the double-bond reduction products **8,9a-f** in good to excellent yields (59-99%). Further functionalizations of 3-bromopyridine derivatives **9a-f** allowed to install various EWGs at C-3 position of the pyridine ring. To this end, a Pd-catalyzed cross-coupling reaction between 3-bromopyridine derivatives **9a-f** and 1-(ethoxyvinyl)tri(*n*-butyl)stannane furnished the desired 3-acetylpyridine derivatives **10a-f** in good yields (60-87%).[47] Alternatively, starting from **9a** and 2,4,6-trichlorophenyl formate, a Pd-catalyzed cross-coupling reaction afforded the corresponding reactive ester **11** in 55% yield. The later was subsequently reacted with various heteronucleophiles to give rise to variously 3-subtituted pyridines **12-14** (75-99% yields). Thereafter, the dehydration reaction of pyridine-3-carboxamide derivative **14** conducted with MSTFA and $CuCl_2$ led to the desired pyridine-3-carbonitrile derivative **15** in 83% yield. Indanones **8**, **10a-f**, **12-15** thus obtained were easily transformed into the corresponding indanone-derived *N*-benzylpyridinium salts **2a-r** (63-99% yields) by quaternization in the presence of the appropriate aryl bromide.

Scheme 1. Synthesis of novel indanone-derived *N*-benzylpyridinium salts **2**[a]



[a]Reagents and conditions: (i) PTSA, toluene, Dean-Stark (26-79%); (ii) H_2, Pt/C, EtOH, 20°C (59-99%); (iii) For **10a-f** from **9a-f**: $Pd(dba)_2$, PPh_3, 1-(ethoxyvinyl)tri(*n*-butyl)stannane then HCl, reflux (60-87%); (iv) For **11** from **9a**: Xantphos, $Pd(OAc)_2$, 2,4,6-trichlorophenyl formate, toluene, Schlenk tube, 110°C, overnight (55%); (v) MeOH, NEt_3, Schlenk tube, 70°C, 20h for **12** (75%) or $MeNH_2$, NEt_3, THF, Schlenk tube, 70°C, 24h for **13** (99%) or NH_3 in dioxane, Schlenk tube, 70°C, 20h for **14** (78%); (vi) $CuCl_2$, MSTFA, toluene (83%); (vii) $ArCH_2Br$, CH_2Cl_2 or ACN, reflux, 12h (63-99%).

In vitro Inhibition Studies of *h*AChE / *eq*BChE and SARs. In addition to AChE, recent studies revealed that BChE may also have a crucial role in the progression of AD.[48-52] While the level of AChE decrease dramatically in the advanced stage of AD, that of BChE is maintained constant or, better still, may be increased in brain regions involved in cognitive functions. These observations lead one to believe that inhibition of both AChE and BChE may contribute to improve current symptomatic treatments of AD. Therefore, both AChE and BChE inhibitory activities of the newly prepared racemic indanone-derived *N*-benzylpyridinium salts **2a-r** was evaluated by means of Ellman's spectrophotometric method[53] using AChE from human erythrocytes and BChE from equine serum. Donepezil and tacrine were used as reference

standards (Table 1). In pyridinium salt **2a**, the substitution pattern of both indanone and benzyl moieties being the same as that of donepezil, this compound was prepared as reference for comparison purpose with donepezil. To our delight, this first pyridinium **2a** displayed an interesting two-digit nanomolar *h*AChE inhibitory activity (IC_{50}=36 nM) comparable to that of donepezil (IC_{50}=11 nM). We then undertook to study the influence of the EWG at C-3 of the pyridine scaffold on *h*AChE inhibitory activity. This stabilizing functional group, essential to ensure good stability of the 1,4-dihydropyridine prodrugs **1**, should nevertheless not disrupt the inhibitory activity of the corresponding pyridinium drugs **2**. This is all the more interesting as, to the best of our knowledge, all SAR investigations regarding donepezil mainly focused on both ends of the molecule (namely indanone and benzyl moieties located at the PAS and CAS respectively), leaving aside the central part of the donepezil which interacts with the active mid gorge.[54]

As depicted in Table 1, a set of indanone-derivated *N*-benzylpyridinium salts **2** bearing at C-3 position a nitrile (**2b**), an acetyl (**2c-l)**, an ester **(2o,p)** and a primary carboxamide (**2q,r**) were evaluated on *h*AChE. Most of them turned out to be highly potent *h*AChEIs, exhibiting one- to two-digit nanomolar IC_{50} values ranging from 2.9 to 91 nM, except for targets **2l** and **2q** whose IC_{50} values dropped to 262 and 678 nM respectively. Of all EWGs surveyed, only secondary carboxamides (**2m,n**) showed no inhibition on *h*AChE (IC_{50} > 10μM), ruling out the use of this class of EWGs as stabilizing element in prodrugs **1**. We then focused our interest on the influence of the substitution pattern at the benzyl moiety by comparing the *h*AChE inhibitory activities of compounds **2c-g** (EWG=COMe; R^2=R^3=OMe). In this series, the benzyl appendage is differently substituted at the ortho- or meta-position by a methyl group or a chlorine atom. Whereas the unsubstituted benzyl derivative **2c** already showed a remarkable *h*AChE inhibitory

activity (IC_{50}=18 nM), the substituted benzyl derivatives **2d-g** prove to be slightly more potent (IC_{50}= 3-14 nM), 3-chlorobenzyl derivative **2g** offering the best result of this series (IC_{50}=3 nM). Lastly, we also briefly looked at the substitution pattern of the indanone ring by comparing the performance in inhibiting *h*AChE of a series of compounds **2d,h-l** (EWG=COMe; Ar=2-MePh) differently substituted at the indanone moiety (R^2= H, OMe, Me; R^3= H, OMe, Me, Cl; R^2= R^3= OCH_2O). In short, IC_{50} values followed the same trend as donepezil;[18] namely compound **2d** bearing a 5,6-dimethoxy indanone ring revealed to be the most potent (IC_{50}= 8 nM). All other compounds **2h-k** displayed a good but lower two-digit nanomolar potency against *h*AChE (IC_{50}= 27-91 nM), while a much lower inhibition was recorded with compound **2l** (IC_{50} =262 nM) highlighting the deleterious effect of the electron withdrawing chlorine atom at the C-5 position. Although donepezil is known to be selective for AChE and to display low affinity for BChE (IC_{50} = 3.3 μM),[18] the inhibitory activity of a large selection of our *N*-benzylpyridinium analogues **2d-m,o,q,r** towards *eq*BChE was evaluated (Table 1). Unsurprisingly, the majority of compounds **2** exhibited a low inhibitory activity against *eq*BChE in the same micromolar range as that measured for donepezil. Interestingly, compound **2j** stands out with a satisfactory IC_{50} value of 262 nM on *eq*BChE together with a high *h*AChE inhibitory activity (IC_{50}=27 nM).

Table 1. Inhibition of AChE from Human Erythrocytes (*h*AChE) and Propidium Iodide Displacement (*Ee*AChE) by the Target Compounds

compd	EWG	R^2	R^3	Ar	IC_{50} (nM) ± SD		Selectivity for AChE[c]	Propidium iodide displacement (%)[d]
					*h*AChE[a]	*eq*BChE[b]		
2a	H	OCH_3	OCH_3	Ph	36.5±4	nd[e]	-	nd[e]

2b	CN	OCH_3	OCH_3	Ph	2.92±0.1	nd[e]	-	25±2
2c	$COCH_3$	OCH_3	OCH_3	Ph	18±1.5	nd[e]	-	26±3
2d	$COCH_3$	OCH_3	OCH_3	2-MePh	8.8±0.2	5930±440	674	14±1
2e	$COCH_3$	OCH_3	OCH_3	3-MePh	14±1.6	5990±510	427	19±1
2f	$COCH_3$	OCH_3	OCH_3	2-ClPh	18±3	1915±145	106	19±1
2g	$COCH_3$	OCH_3	OCH_3	3-ClPh	3.27±0.1	3340±180	1021	16±1
2h	$COCH_3$	OCH_2O	-	2-MePh	60±3	1615±45	26.9	19±2
2i	$COCH_3$	H	OCH_3	2-MePh	30±1	2990±240	133	23±3
2j	$COCH_3$	CH_3	CH_3	2-MePh	27.7±3.7	262±15	6.95	18±2
2k	$COCH_3$	H	CH_3	2-MePh	91±9	3815±375	41.9	20±2
2l	$COCH_3$	H	Cl	2-MePh	262±17	3630±90	13.9	20±3
2m	$CONHCH_3$	OCH_3	OCH_3	Ph	>10μM	4765±425	-	nd[e]
2n	$CONHCH_3$	OCH_3	OCH_3	2-MePh	>10μM	nd[e]	-	nd[e]
2o	$COOCH_3$	OCH_3	OCH_3	Ph	25.6±4.7	3055±65	119	16±0
2p	$COOCH_3$	OCH_3	OCH_3	2-MePh	5.8±0.2	1792±170	309	nd[e]
2q	$CONH_2$	OCH_3	OCH_3	2-MePh	678±42	>10μM	-	16±1
2r	$CONH_2$	OCH_3	OCH_3	3-ClPh	41±20	>10μM	-	20±4
donepezil					6.4±0.4 or 11[18]	3365[17]	306	17±1
tacrine					45.1±7[55]	3.2±0.2		

[a]The 50% inhibitory concentration (means ± SD of three expermiments) of AChE from human erythrocytes. [b]The 50% inhibitory concentration (means ± SD of three experiments) of BChE from equine serum. [c]Selectivity for AChE = IC_{50} (*eq*BChE)/IC_{50} (*h*AChE). [d]Propidium iodide displacement conducted on *Ee*AChE by the tested compound and donepezil as control at 1.0μM. [e]Not determined.

Inhibition of the Peripheral Anionic Site (PAS), kinetic studies and anti-amyloid aggregation activity. The affinity of AChEIs **2** for the PAS of AChE was examined at 1.0μM concentration using the method of Taylor et al.[56] which implements displacement studies with propidium iodide, a known PAS-specific ligand of AChE. The results obtained in Table 1 showed that most of the tested drugs **2** were slightly more efficient to displace propidium (18-

26%) than donepezil (17%), with the exception of **2d,o** which appeared to be somewhat less potent (12-16%). To gain further insight into the mechanism of AChE enzyme inhibition and to ascertain the dual-binding site character of this new class of AChEIs **2**, an enzyme kinetic study was performed on selected drug **2r** using *h*AChE. As explained bellow, the better solubility of the corresponding prodrug **1r** prompted us to pursue our in vitro biological investigation with **2r**. The Lineweaver-Burk double reciprocal plots were constructed with a range of substrate concentrations as depicted in Fig. 3. Analysis of the plots revealed that by increasing the concentrations of **2r**, an increase in slope occurred (reflecting a reduction of V_{max}) whereas K_m remains unchanged. These two findings are consistent with a non-competitive inhibition of AChE, thus supporting the dual binding character of **2r** that binds, in all likelihood, to both CAS and PAS of the enzyme. A thioflavin T-based fluorometric assay[57] was accomplished with the dual binding site inhibitor **2r** to ascertain whether inhibition of the PAS may prevent Aβ peptide aggregation. Gratifyingly, **2r** inhibits AChE-induced Aβ1–40 aggregation by 41±2 % at the concentration of 100 μM, compared to 75±2% with propidium iodide as reference standard under the same conditions. In addition, although **2r** exhibits moderate inhibition of self-induced $A\beta_{1-42}$ aggregation (25%±3) at 10 μM concentration, it is worth noting that donepezil displays a lower anti-aggregation activity (13%) at the same concentration.[58]

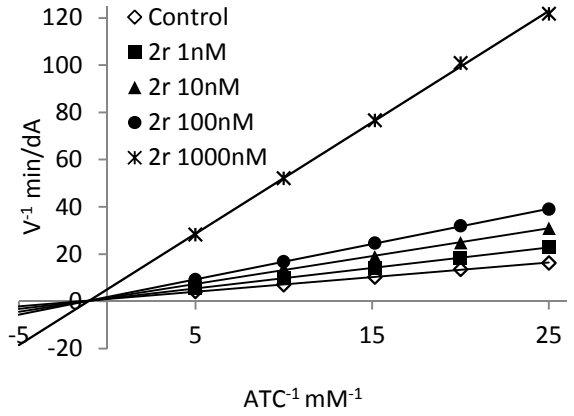
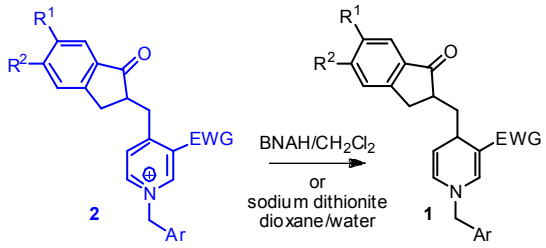


Figure 3. Kinetics study on the mechanism of *h*AChE inhibition by **2r**. Lineweaver−Burk reciprocal plots of the AChE initial velocity at increasing substrate concentrations (0,04-0,2 mM) in the absence and presence of **2r** (1-1000 nM) are represented. ATC = acetylthiocholine; V = initial velocity rate.

In vitro evaluation of the donepezil-based "bio-oxidizable" prodrug system. After having demonstrated the potential of indanone-derived *N*-benzylpyridinium salts **2** to act as highly potent dual binding site AChEIs, it remained to be established whether the corresponding 1,4-dihydropyridines **1** are inactive or much less active against AChE to be considered as "bio-oxidizable" prodrug candidates. To this end, regioselective reduction of selected indanone-derived *N*-benzylpyridinium salts **2d,h-l,q,r** afforded the corresponding 1,4-dihydropyridines **1d,h-l,q,r** as a diastereoisomeric mixture which were subsequently evaluated for their inhibitory activity against *h*AChE (Table 2, 35-59% yields). Pleasingly, most of the dihydropyridines **1** proved to be inactive against AChE (IC_{50}>1 μM), with the exception of **1d,j** which revealed a weak activity (IC_{50} = 428nM and 653 nM respectively), however much lower than that of the parent pyridinium salts **2d,j** (IC_{50} = 8.8 nM and 27.7 nM respectively).

Table 2. Inhibition of AChE from Human Erythrocytes (*h*AChE) by Selected Prodrugs **1**



compd	EWG	R^1	R^2	Ar	IC_{50} (nM)±SD *h*AChE[a]
1d	$COCH_3$	OCH_3	OCH_3	2-MePh	428±42
1h	$COCH_3$	OCH_2O	-	2-MePh	>1μM
1i	$COCH_3$	H	OCH_3	2-MePh	>1μM
1j	$COCH_3$	CH_3	CH_3	2-MePh	653±39
1k	$COCH_3$	H	CH_3	2-MePh	>1μM
1l	$COCH_3$	H	Cl	2-MePh	>1μM
1q	$CONH_2$	OCH_3	OCH_3	2-MePh	>1μM
1r	$CONH_2$	OCH_3	OCH_3	3-ClPh	>1μM

A crucial task of the "bio-oxidizable" prodrug **1** consists in crossing the BBB to deliver the active drug **2** into the brain after a subsequent redox-activation step. The aptitude of 1,4-dihydropyridines **1h,i,r** and their corresponding pyridinium salts **2h,i,r** to cross the BBB by passive diffusion were then evaluated using an in vitro parallel artificial membrane permeability assay of blood-brain barrier (PAMPA-BBB). Permeability values P_e (10^{-6}cm/s) for the prodrug **1**/drug **2** systems tested at 100 μM in a buffer pH 7.4, were measured to be 14.3±1.5/0±0 (**1h**/**2h**), 14.8±0.6/0.3±0.2 (**1i**/**2i**), 25.6±2.3/0.15±0.07 (**1r**/**2r**). On the basis of these results, it can be reasonably concluded that 1,4-dihydropyridines **1h,i,r** are lipophilic enough to reach the

brain, as their P_e values are well above the threshold predicting high BBB permeability ($P_e >$ 5.2). As might be expected, the corresponding quaternary pyridinium salts **2h,i,r** would not penetrate the BBB by passive diffusion suggesting that once in the brain, AChEIs **2** will be subjected to a "locked-in" effect to exhibit a central selective AChE inhibition. For this scenario to be successful, prodrugs **1** should be stable enough to ensure that any early conversion to the parent AChEIs **2** occurs at the periphery, while being able to undergo rapid oxidation once in the brain to thwart competing metabolisms and to trap the permanently charged AChEIs **2** within the brain tissue. To gain insights into this activation step of prodrugs **1**, the rate and the nature of the oxidation was investigated by incubation in various oxidative media ($AgNO_3$, NAD^+ and riboflavin solutions, mice brain homogenate). For solubility reasons, we decided to focus our study on the 1,4-dihydropyridine/pyridinium salt couple **1r**/**2r** as "bio-oxidizable" prodrug system. First, we evaluated the in vitro stability of prodrug **1r** in PBS and in human plasma at 37°C (Fig. 4). After 3 hours, only traces of oxidation product **2r** could be detected without any other degradation product, allowing us to foresee a good stability of **1r** at the periphery. In the presence of $AgNO_3$, a significant amount of oxidation product **2r** was detected after 3 hours. Although a chemical oxidation route can occur, an enzymatic activation step of the prodrug **1r** cannot be ruled out, all the more so as oxidation of dihydropyridines by NADH dehydrogenase has already been reported.[59] A relevant observation that argues in favor of this hypothesis is the marked acceleration in the rate of oxidation of prodrug **1r** when using a 2% NAD^+ or a 2% riboflavin solution. It is worth noting that NAD^+ and riboflavin act respectively as coenzyme and prosthetic group precursor of NADH dehydrogenase. Finally yet importantly, whereas incubation of prodrug **1r** conducted in 10% fresh mice brain homogenate led to the oxidation product **2r** (35%), the use of mice brain homogenate stored beforehand for 3 months at -20°C failed to

furnish **2r**. This time-dependent loss of enzyme activity is the hallmark of NADH hydrogenase and therefore consistent with the involvement of this enzymatic system in the oxidation of prodrug **1r** (Figure 3).

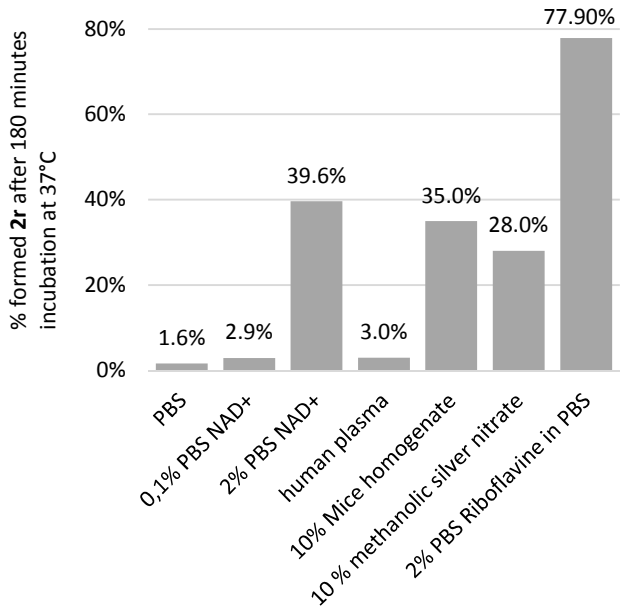


Figure 4. Activation of the "bio-oxidizable" prodrug **1r** in various oxidative media.

Another appealing aspect of our "bio-oxidizable" prodrug approach arises from the possibility for 1,4-dihydropyridines **1** to exhibit radical scavenging and antioxidant properties during its conversion to the corresponding AChEIs **2**. The antioxidant activity of prodrug **1r** was therefore assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method,[60-62] one of the most frequently used assay for evaluating in vitro antioxidant activity by measuring the ability of tested compounds to act as free radical scavengers or hydrogen donors. From this assay, it was observed that prodrug **1r** exhibits a noticeable scavenging activity (EC_{50} = 90 μM) whereas donepezil does not show any activity. Furthermore, a set of NMR experiments clearly demonstrated that prodrug **1r** reacts with the stable radical DPPH$^{\cdot}$ to produce the desired AChEI

2r as the main oxidation product (see supporting information). These findings suggest that central conversion of prodrug **1r** to AChEI **2r** may possibly lead to additional antioxidant properties with potential protective effects against oxidative stress.

Genotoxicity tests are now recommended at earlier stages of drug discovery to reduce the failure probability in advanced stage of development. Therefore, the genotoxicity of compounds **1r** and **2r** was evaluated by the Ames fluctuation test from Toxem (France). The genotoxic activity was determined using *Salmonella thyphimurium* TA98 and TAMix (mixture of six base pair mutant strains TA7001-TA7006) with and without metabolic activation. Satisfactorily, compounds **1r** and **2r** did not induce significant number of revertant colonies both in presence and in absence of metabolic activation.

We also investigated the cytotoxicity profile of pyridinium compound **2r** on human neuroblastoma SH-SY5Y cell lines using a Calcein-AM assay. As a control, the neuroblastoma cells were also treated with donepezil and the known neurotoxin 1-methyl-4-phenylpyridinium (MPP+).[63] Cells were treated with compound **2r**, donepezil and MPP+ at various concentrations (0.1 to 1 mM) for 24h prior to measuring cell viability. In contrast to MPP+ and donepezil for which a dose-related effect was clearly observed, we are pleased to note that compound **2r** did not induced toxicity in all tested doses on the SH-SY5Y cells (see supporting information).

Docking Study of 1r and 2r with *h*AChE. To gain insight into binding interactions of AChEI in the hydrolytic active site, we carried out molecular docking studies of **1r** and **2r** compounds. These studies were performed into a human AChE structure (PDB ED: 4EY7)[28] and during the docking a water molecule interacting with protonated piperidine ring of donepezil was conserved (residue number 931) and the protonation schema proposed by proPKA software was applied on

AChE (Glu202 protonate, His447 protonate at δ position...). (*R*)- and (*S*)-enantiomers of **2r** and (*R*,*R*)- and (*S*,*S*)-enantiomers of **1r** were docked independently in the active site using the GOLD program and applying ChemPLP scoring function. Both enantiomers took a similar position close to donepezil in the crystal structure of *h*AChE/donepezil complex (Figure 5C).[28] The visualization of AChE binding site surface showed two cavities having the possibility to accommodate the EWG group, the first in W86 proximity and the second one in His447 proximity (Figure 4D). The docking placed the EWG group of **1r** and **2r** systematically in the His447 cavity. The docking results suggest that, as donepezil, the indanone moiety of **1r** and **2r** are situated in W286 proximity of the Peripheric binding site (PAS) well positioned for π-stacking. *N*-benzyl group stacks above W86 of anionic hole and the indanone carbonyl group establishes an H-bond with Phe295 backbone NH. The cyclic nitrogen atom of both ligands takes a position suitable for an electrostatic interaction with the water molecule in the proximity of Tyr337 and Tyr341. According to the docking results, all key interactions are preserved by **1r** and **2r**. However, **1r** and **2r** exhibit very different inhibition activities. As the unique difference between both compounds lies in the presence of the pyridinium ion in **2r**, we assumed that the positively charged nitrogen atom is necessary to display high AChE inhibition activity. Finally, we were also very concerned about the difference on *h*AChE inhibition observed between the secondary amide group in **2m,n** and ester function in **2o,p**. So, docking studies were also performed on **2n** and **2p**. Interestingly, the ester EWG in **2p** for which the oxygen atom from the OMe group is in front of His447 HN_{δ} (see supporting information) can give interactions and is then in agreement with the measured inhibition activities. On the contrary, the NH part of the secondary amide group of **2n** was placed in front of HN_{δ} of His447 which is not suitable for an

interaction. This result is thus consistent with the observed ineffectiveness of **2n** on the *h*AChE inhibition (>10μM).

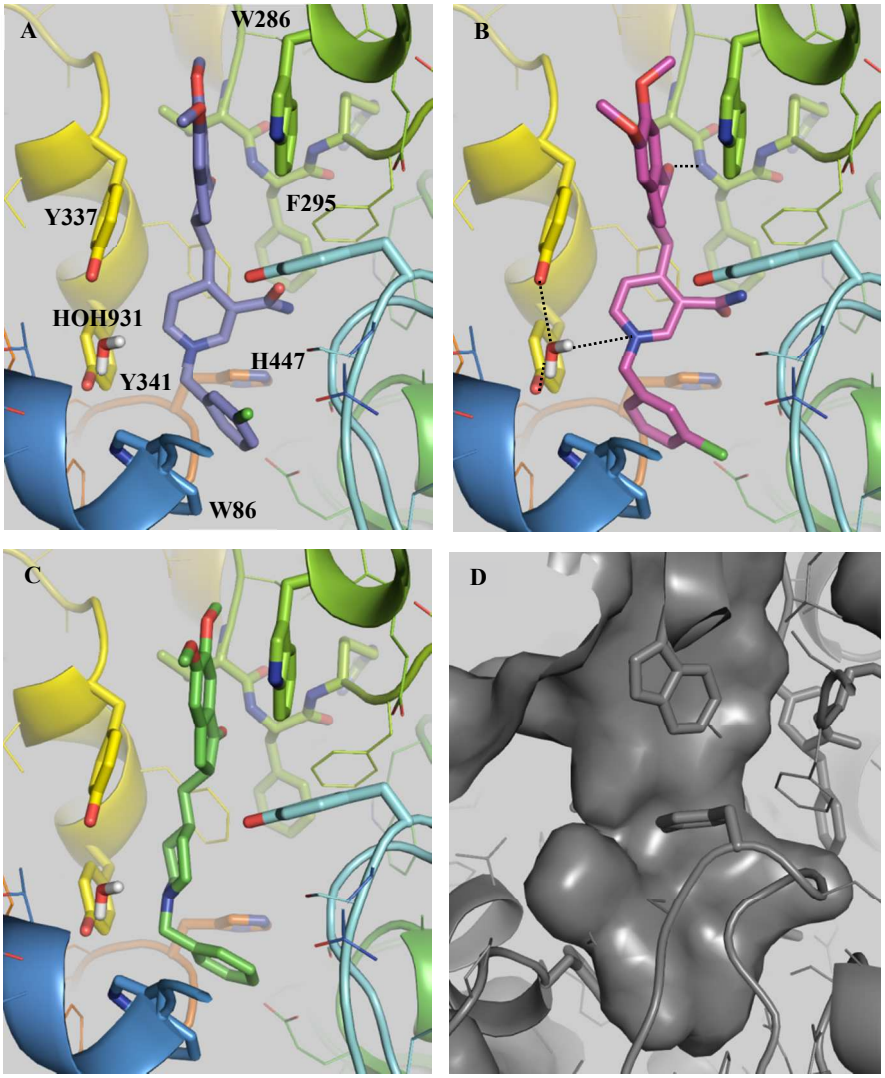


Figure 5. Dihydropyridine (*S,S*)-**1r** (A) and pyridinium (*S*)-**2r** (B) positioned in AChE binding sites using the docking studies, compared to the donepezil position from the X-ray structure (C) as well as the binding cavity surface (D). The compound and the selected side chains of the binding site residues are in stick and the protein in ribbon representation. This figure was made with PYMOL (DeLano Scientific, 2002, San Carlo, USA).

In vivo toxicity evaluation of the donepezil-based prodrug system. Finally, preliminary toxicological experiments were accomplished to provide a first insight in the toxicological profiles of the selected compounds **1r** and **2r**. Donepezil and rivastigmine were used as reference drugs. The median lethal dose (LD_{50}) is a helpful pointer of the substance's acute toxicity. The LD_{50} dose was established after intraperitoneal injection in female CD-1 IGS mice 20-22g (Charles River Laboratories France) followed by an observation period of 48 hours. The LD_{50} values in mg/kg were determined to be as follows: for **1r** (>50 solubility limit), **2r** (70±10), donepezil (22.5±1.5) and rivastigmine (6.2±0.6). These results point out a low acute toxicity of the prodrug/drug system **1r**/**2r** with particular high LD_{50} values compared with reference drugs. The potential toxicity of compound **1r** was then evaluated following once daily intraperitoneal administration (10 mg/kg) in mice for 14 days. At this dose level, the compound **1r** did not induce mortality. A slight freezing was observed with only one animal. This sign appeared within 30 minutes to 1h after treatment the first 3 days and was not observed thereafter until the end of the treatment. No other clinical signs were observed during the treatment period. Compared to control group, a slight weight loss was observed in 4/5 animals during the first three days after the start of treatment with **1r** but was discontinued until the day 14. After necroscopy, principal organs (brain, heart, kidney, liver, spleen…) were submitted to a macroscopic and microscopic examination. At the studied dose, compound **1r** did not induce changes on the controlled organs. To ensure that no accumulation of **2r** in the brain occurs after repeated daily administration, ex vivo analysis of brain tissue after 24h and 14 days were performed (by LC MSMS) and no significant quantities of **2r** were detected.

CONCLUSION

Although the search of curative treatments for AD has long been, and is still a priority, improvement of currently prescribed symptomatic treatments remains an important field of research. These symptomatic treatments, which mainly rely on AChEIs to restore the cholinergic balance in brain of AD patients in order to preserve cognitive functions, are often associated with serious side effects attributable to peripheral cholinergic stimulation. With the ultimate goal to prevent these deleterious adverse effects, we reported herein a "bio-oxidizable" prodrug approach by taking a cue from Bodor's redox chemical delivery system to develop new central AChEIs based on the standard drug donepezil. Thus, a set of indanone-derived *N*-benzylpyridinium salts **2** and their corresponding "bio-oxidizable" prodrug 1,4-dihydropyridines **1** were prepared and evaluated in vitro. Our study provides in vitro proof-of-concept of this "bio-oxidizable" prodrug strategy by validating the main features required; namely (1) high inhibitory activity of indanone-derived *N*-benzylpyridinium salts **2** towards AChE; (2) dual binding site mode of interactions by binding to both the CAS and PAS of AChE leading to anti-amyloid aggregation activity; (3) any relevant inhibitory activity of the corresponding indanone-derived *N*-benzyl 1,4-dihydropyridines **1** against AChE; (4) good prediction of BBB penetration for prodrugs **1** and brain trapping of the corresponding permanently charged AChEIs **2**; (5) good stability of the prodrug **1r** in human plasma and smooth conversion back to the parent drug **2r** under various mild oxidizing conditions, not to mention promising antioxidant properties of prodrug **1r**. Finally, both compounds **1r** and **2r** did not exhibit genotoxicity from Ames tests and showed relatively low acute toxicity in mice, offering the prospect of further in vivo biological developments.

EXPERIMENTAL SECTION

Chemistry. All commercial reagents were used without further purification. The solvents were dried with appropriate desiccants and distilled prior to use or were obtained anhydrous from commercial suppliers. Silica gel (60, 230–400 mesh or 70–230 mesh) was used for column chromatography. Reactions were monitored by thin layer chromatography on silica gel precoated aluminium plates. UV light at 254 nm or $KMnO_4$ stains were used to visualize TLC plates. ^1H, ^{13}C NMR spectra were recorded using a spectrometer operating at 300 and 75 MHz respectively. Abbreviations used for peak multiplicities are s: singlet, d: doublet, t: triplet, q: quadruplet dd = doublet of doublet, br = broad and m: multiplet. Coupling constants *J* are in Hz and chemical shifts are given in ppm and calibrated with DMSO-d_6 or $CDCl_3$ (residual solvent signals). ^1H NMR spectra obtained in $CDCl_3$ were referenced to 7.26 ppm. ^{13}C NMR spectra obtained in $CDCl_3$ were referenced to 77.16 ppm and in DMSO-d_6 were referenced to 39.52 ppm. Elemental analyses were performed by the microanalysis service of the University of Rouen and were recorded with a Thermo Scientific FLASH 2000 analyzer.

General procedure A for synthesis of compounds 1d, 1h, 1i, 1j, 1k, 1l. To a solution of pyridinium salt **2** (0.2 mmol) in CH_2Cl_2 (6 mL) was added *N*-benzyl-1,4-dihydronicotinamide (BNAH) (0.2 mmol, 1 equiv) at room temperature under Argon. The resulting solution was stirred in darkness until completion of the reaction (TLC). The reaction mixture was then washed with H_2O (2x) and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography on silica gel of the crude residue afforded compounds **1** as a diastereomeric mixture.

*2-[[3-Acetyl-1-(o-tolylmethyl)-4H-pyridin-4-yl]methyl]-5,6-dimethoxy-indan-1-one (**1d**)*. The title compound **1d** was prepared according to the general procedure A with compound **2d** (76.2 mg, 0.15 mmol) and BNAH (32.1 mg, 1 equiv) as reactants. Rf = 0.50/ 0.59 (100% EtOAc). Pale

brown solid. (Yield 32.3 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.35-1.46 (m, 0.55H), 1.48-1.60 (m, 0.45H), 1.88-2.09 (m, 1H), 2.12 (s, 1.65H), 2.18 (s, 1.35H), 2.29 (s, 3H), 2.63-2.85 (m, 1.45H), 3.07 (dd, 0.55H, *J* = 17.1 Hz, *J* = 3.3 Hz), 3.17-3.35 (m, 1H), 3.58-3.66 (m, 0.55H), 3.69-3.79 (m, 0.45H), 3.88 (s, 3H), 3.94-3.95 (m, 3H), 4.44 (s, 2H), 4.95 (dd, 0.45H, *J* = 7.8 Hz, *J* = 5.4 Hz), 5.12 (dd, 0.55H, *J* = 7.8 Hz, *J* = 5.1 Hz), 5.89-5.96 (m, 1H), 6.84 (s, 0.45H), 6.88 (s, 0.55H), 7.06-7.28 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.2, 24.5, 24.6, 29.2, 29.4, 32.6, 33.8, 40.5, 41.4, 44.1, 45.2, 56.0, 56.1, 104.2, 107.4, 107.6, 108.3, 110.0, 113.1, 113.8, 126.6, 127.6, 128.2, 129.4, 130.9, 134.4, 136.1, 142.7, 143.3, 149.3, 149.7, 149.8, 155.3, 155.4, 194.9, 195.5, 208.4, 208.5. Anal. Calcd. for $C_{27}H_{29}NO_4$: C, 75.15; H, 6.77; N, 3.25. Found: C, 75.37; H, 6.89; N, 3.21.

*6-[[3-Acetyl-1-(o-tolylmethyl)-4H-pyridin-4-yl]methyl]-5,6-dihydrocyclopenta[f][1,3]benzodioxol-7-one (**1h**).* The title compound **1h** was prepared according to the general procedure A with compound **2h** (100 mg, 0.20 mmol) and BNAH (43 mg, 1 equiv) as reactants. Pale brown solid. (Yield 32.1 mg, 38%). Rf = 0.46/ 0.61 (100% EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.34-1.59 (m, 0.54H), 1.86-2.00 (m, 0.46H), 2.13 (s, 1.6H), 2.18 (s, 1.4H), 2.31 (2s, 3H), 2.64-2.86 (m, 1.46H), 3.03 (dd, 0.54H, *J* = 17.1 Hz, *J* = 3.3 Hz), 3.15-3.32 (m, 1H), 3.56-3.65 (m, 0.54H), 3.70-3.79 (m, 0.46H), 4.43 (s, 2H), 4.96 (dd, 0.44H, *J* = 7.5 Hz, *J* = 5.6 Hz), 5.10 (dd, 0.56H, *J* = 7.5 Hz, *J* = 5.1 Hz), 5.91-5.96 (m, 1H), 6.04 (brs, 2H), 6.79 (s, 0.46H), 6.82 (s, 0.54H), 7.05-7.28 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.2, 19.3, 24.5, 24.6, 29.2, 29.3, 32.8, 34.1, 40.5, 41.1, 44.3, 45.3, 56.0, 102.1, 102.3, 105.7, 105.9, 108.2, 109.9, 113.1, 113.8, 126.6, 126.7, 127.6, 127.7, 128.2, 130.9, 131.2, 134.4, 136.1, 142.7, 143.3, 148.1, 151.8, 151.9, 154.2, 154.3, 194.9, 195.4, 207.5, 207.6. HRMS (ESI^+) *m/z*

416.1844 $[M+H]^+$, calcd for $C_{26}H_{26}NO_4$ 416.1862. Anal. Calcd. for $C_{26}H_{25}NO_4$: C, 75.16; H, 6.06; N, 3.37. Found: C, 75.31; H, 6.14; N, 3.41.

*2-[[3-Acetyl-1-(o-tolylmethyl)-4H-pyridin-4-yl]methyl]-5-methoxy-indan-1-one (**1i**).* The title compound **1i** was prepared according to the general procedure A with compound **2i** (100 mg, 0.21 mmol) and BNAH (45 mg, 1 equiv) as reactants. Yellow solid. (Yield 34.5 mg, 41%). Rf = 0.52/0.60 (100% EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.38-1.59 (m, 0.54H), 1.83-2.03 (m, 0.46H), 2.12 (s, 1.6H), 2.17 (s, 1.4H), 2.29 (2s, 3H), 2.61-2.88 (m, 1.46H), 3.12 (dd, 0.54H, *J* = 17.4 Hz, *J* = 3.6 Hz), 3.19-3.39 (m, 1H), 3.56-3.66 (m, 0.54H), 3.72-3.88 (m, 0.46H), 3.86 (s, 3H), 4.43 (s, 2H), 4.95 (dd, 0.44H, *J* = 7.8 Hz, *J* = 5.7 Hz), 5.10 (dd, 0.56H, *J* = 7.8 Hz, *J* = 5.4 Hz), 5.89-5.94 (m, 1H), 6.84-6.88 (m, 2H), 7.07-7.28 (m, 5H), 7.61-7.65 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.2, 24.5, 24.6, 29.2, 29.3, 32.9, 34.2, 40.4, 41.2, 44.0, 45.0, 55.6, 56.0, 108.3, 109.6, 109.7, 109.9, 113.1, 113.8, 115.2, 115.4, 125.4, 126.6, 126.7, 127.6, 127.7, 128.2, 130.0, 130.1, 134.4, 136.0, 142.8, 143.3, 157.4, 157.5, 165.2, 165.3, 194.9, 195.5, 207.8, 207.9. HRMS (ESI^+) *m/z* 402.2053 $[M+H]^+$, calcd for $C_{26}H_{28}NO_3$ 402.2069. Anal. Calcd. for $C_{26}H_{27}NO_3$: C, 77.78; H, 6.78; N, 3.48. Found: C, 77.9; H, 6.85; N, 3.45.

*2-[[3-Acetyl-1-(o-tolylmethyl)-4H-pyridin-4-yl]methyl]-5,6-dimethyl-indan-1-one (**1j**).* The title compound **1j** was prepared according to the general procedure A with compound **2j** (62.0 mg, 0.13 mmol) and BNAH (33 mg, 1 equiv) as reactants. Yellow solid. (Yield 20.0 mg, 38%). Rf = 0.7/0.78 (100% EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.37-1.61 (m, 0.55H), 1.89-1.99 (m, 0.45H), 2.12 (s, 1.6H), 2.18 (s, 1.4H), 2.25-2.36 (m, 9H), 2.61-2.86 (m, 1.45H), 3.05 (dd, 0.55H, *J* = 17.1 Hz, *J* = 3.3 Hz), 3.17-3.37 (m, 1H), 3.59-3.66 (m, 0.55H), 3.72-.379 (m, 0.45H), 4.44 (s, 2H), 4.95 (dd, 0.45H, *J* = 7.5 Hz, *J* = 5.4 Hz), 5.10 (dd, 0.55H, *J* = 7.5 Hz, *J* = 5.1 Hz), 5.89-5.95 (m, 1H), 7.07 (s, 0.55H), 7.12-7.45 (m, 5.45H), 7.50 (s, 1H). ^{13}C NMR (75 MHz,

$CDCl_3$, δ): 19.2, 19.3, 19.8, 20.8, 24.5, 24.6, 29.3, 29.4, 32.4, 33.7, 40.4, 41.0, 44.0, 45.1, 56.0, 108.2, 109.9, 113.2, 113.9, 124.1, 126.6, 127.3, 127.5, 127.6, 128.2, 130.9, 134.4, 134.9, 136.1, 142.7, 143.2, 144.7, 144.8, 152.5, 152.6, 194.9, 195.4, 209.5, 209.6. HRMS (ESI^+) *m/z* 400.2263 $[M+H]^+$, calcd for $C_{27}H_{30}NO_2$ 400.2277. Anal. Calcd. for $C_{27}H_{29}NO_2$: C, 81.17; H, 7.32; N, 3.51. Found: C, 81.38; H, 7.35; N, 3.54.

*2-[[3-Acetyl-1-(o-tolylmethyl)-4H-pyridin-4-yl]methyl]-5-methyl-indan-1-one (**1k**).* The title compound **1k** was prepared according to the general procedure A with compound **2k** (150 mg, 0.32 mmol) and BNAH (69 mg, 1 equiv) as reactants. Pale yellow solid. (Yield 43.5 mg, 35%). Rf = 0.69/ 0.74 (100% EtOAc). ^1H NMR (300 MHz, $CDCl_3$, δ): 1.38-1.58 (m, 0.56H), 1.89-1.96 (m, 0.44H), 2.12 (s, 1.6H), 2.17 (s, 1.4H), 2.30 (s, 3H), 2.41 (s, 3H), 2.62-2.71 (m, 0.44H), 2.72-2.88 (m, 1H), 3.05-3.45 (m, 1.56H), 3.56-3.66 (m, 0.56H), 3.72-3.76 (m, 0.44H), 4.44 (s, 2H), 4.95 (dd, 0.44H, *J* = 7.8 Hz, *J* = 5.7 Hz), 5.10 (dd, 0.56H, *J* = 7.5 Hz, *J* = 5.1 Hz), 5.90-5.94 (m, 1H), 7.07-7.28 (m, 7H), 7.58-7.61 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.2, 19.3, 22.1, 24.5, 24.6, 29.2, 29.3, 32.7, 34.0, 40.3, 41.0, 44.0, 45.0, 56.0, 108.2, 109.9, 113.2, 113.8, 123.6, 126.6, 126.7, 126.9, 127.1, 127.6, 128.2, 128.5, 130.9, 134.4, 134.5, 136.1, 142.7, 143.3, 145.7, 145.8, 154.9, 155.0, 194.9, 195.4, 209.3, 209.4. HRMS (ESI^+) *m/z* 386.2108 $[M+H]^+$, calcd for $C_{26}H_{28}NO_2$ 386.2120. Anal. Calcd. for $C_{26}H_{27}NO_2$: C, 81.01; H, 7.06; N, 3.63. Found: C, 81.22; H, 7.15; N, 3.62.

*2-[[3-Acetyl-1-(o-tolylmethyl)-4H-pyridin-4-yl]methyl]-5-chloro-indan-1-one (**1l**).* The title compound **1l** was prepared according to the general procedure A with compound **2l** (42 mg, 0.08 mmol) and BNAH (19 mg, 1 equiv) as reactants. Yellow solid. (Yield 17.5 mg, 50%). Rf = 0.60/0.71 (100% EtOAc). ^1H NMR (300 MHz, $CDCl_3$, δ): 1.38-1.59 (m, 1.54H), 1.88-2.01 (m, 1.46H), 2.13 (s, 1.6H), 2.17 (s, 1.4H), 2.29 (s, 3H), 2.64-2.75 (m, 1.46H), 3.12 (dd, 0.54H, *J* =

17.7 Hz, *J* = 3.9 Hz), 3.20-3.43 (m, 1H), 3.58-3.66 (m, 0.54H), 3.71-3.79 (m, 0.46H), 4.44 (s, 2H), 4.94 (dd, 0.44H, *J* = 7.5 Hz, *J* = 5.4 Hz), 5.08 (dd, 0.56H, *J* = 7.8 Hz, *J* = 5.1 Hz), 5.89-5.97 (m, 1H), 7.08-7.28 (m, 5H), 7.41-7.44 (m, 1H), 7.61-7.66 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.2, 19.3, 24.5, 24.6, 29.1, 29.2, 29.8, 32.7, 33.9, 40.2, 40.9, 44.1, 45.1, 56.0, 108.1, 113.0, 109.9, 113.7, 124.9, 126.6, 126.7, 126.8, 127.6, 127.0, 127.6, 127.7, 128.0, 128.1, 128.3, 128.4, 130.9, 134.3, 135.2, 136.1, 141.0, 141.2, 142.8, 143.3, 155.8, 155.9, 194.9, 195.5, 208.2, 208.3. HRMS (ESI^+) *m/z* 406.1568 $[M+H]^+$, calcd for $C_{25}H_{25}ClNO_2$ 406.1574. Anal. Calcd. for $C_{25}H_{24}ClNO_2$: C, 73.97; H, 5.96; N, 3.45. Found: C, 74.21; H, 5.99; N, 3.46.

General procedure B for synthesis of compounds 1q and 1r. To a solution of pyridinium salt **2** (0.25 mmol) in a mixture of dioxane/water (8 mL, 1/1) was added potassium carbonate (1.5 mmol, 6 equiv) and sodium dithionite (1.5 mmol, 6 equiv) at room temperature. The resulting mixture was stirred at 50°C until completion of the reaction (TLC). After cooling at room temperature and adding glacial acetic acid to reach pH 6 and phase separation, the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with water and brine, dried ($MgSO_4$) and concentrated under vacuum. The crude residue was purified by flash chromatography on silica gel to afford compounds **1** as a diastereomeric mixture.

*4-[(5,6-Dimethoxy-1-oxo-indan-2-yl)methyl]-1-(o-tolylmethyl)-4H-pyridine-3-carboxamide (**1q**).* The title compound **1q** was prepared according to the general procedure B with compound **2q** (100.0 mg, 0.196 mmol) as reactant. Pale yellow solid. (Yield 50.0 mg, 59%). Rf = 0.48 (EtOAc/MeOH = 98:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.65-1.67 (m, 0.45H), 1.85-1.96 (m, 0.55H), 2.27-2.28 (m, 3H), 2.63-2.68 (m, 1.45H), 2.69-2.72 (m, 0.45H), 2.97-3.03 (m, 0.55H), 3.23-3.31 (m, 1.55H), 3.63-3.65 (m, 0.45H), 3.82-3.95 (m, 6.45H), 4.37-4.43 (m, 2H), 4.82 (dd, 0.55H, *J* = 7.5 Hz, *J* = 5.7 Hz), 5.00 (dd, 0.45H, *J* = 7.8 Hz, *J* = 5.1 Hz), 5.90 (d, 2H, *J* = 7.8 Hz),

6.84 (s, 1H), 7.12-7.35 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.3, 29.5, 31.4, 34.2, 34.5, 41.8, 42.5, 42.9, 43.8, 55.8, 56.0, 56.2, 56.4, 103.2, 103.6, 104.2, 104.3, 105.9, 106.3, 107.4, 126.4, 128.0, 128.8, 128.9, 129.1, 129.2, 130.7, 135.0, 136.2, 139.8, 140.3, 149.3, 149.6, 156.0, 170.3, 171.0, 209.4, 209.7. HRMS (ESI^+) *m/z* 433.2115 $[M+H]^+$, calcd for $C_{26}H_{29}N_2O_4$ 433.2127. Anal. Calcd. for $C_{26}H_{28}N_2O_4$: C, 72.20; H, 6.53; N, 6.48. Found: C, 72.41; H, 6.57; N, 6.51.

*1-[(3-Chlorophenyl)methyl]-4-[(5,6-dimethoxy-1-oxo-indan-2-yl)methyl]-4H-pyridine-3-carboxamide (**1r**).* The title compound **1r** was prepared according to the general procedure B with compound **2r** (265.9 mg, 0.5 mmol). Pale brown solid. (Yield 110.1 mg, 48%). Rf = 0.46 (EtOAc/MeOH = 98:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.61-1.69 (m, 0.45H), 1.85-1.93 (m, 0.55H), 2.53-2.69 (m, 1H), 2.73-2.85 (m, 0.55H), 2.91-2.99 (m, 0.45H), 3.19-3.27 (m, 1H), 3.57-3.63 (m, 0.45H), 3.84-3.93 (m, 6.55H), 4.33-4.38 (m, 2H), 4.80 (dd, 0.55H, *J* = 7.5 Hz, *J* = 5.7 Hz), 4.98 (dd, 0.45H, *J* = 7.8 Hz, *J* = 5.1 Hz), 5.87-5.92 (m, 1H), 5.99 (br s, 1H), 6.20 (br s, 1H), 6.82 (s, 1H), 7.05-7.35 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 29.3, 31.2, 34.2, 34.4, 41.5, 42.2, 42.8, 43.7, 56.2, 56.3, 57.1, 57.2, 103.8, 104.2, 104.3, 104.4, 106.3, 106.9, 107.4, 125.3, 127.2, 128.1, 128.7, 129.8, 129.0, 129.1, 130.3, 134.8, 139.3, 139.6, 139.9, 149.3, 149.6, 155.8, 156.0, 170.0, 170.7, 209.3, 209.6. HRMS (ESI^+) *m/z* 453.1588 $[M+H]^+$, calcd for $C_{25}H_{26}ClN_2O_4$ 453.1581. Anal. Calcd. for $C_{25}H_{25}ClN_2O_4$: C, 66.29; H, 5.56; N, 6.18. Found: C, 66.41; H, 5.62; N, 6.20.

General procedure C for synthesis of compounds 2a-r. Compounds **8**, **10a-e, 12-15** (0.2 mmol) were dissolved in dichloromethane (2 mL). Benzyl bromide derivative (1.2 to 2.0 equiv) was then added and the solution was heated under reflux for 12 h in a sealed tube. After

concentration under reduced pressure, the solid was triturated in diethyl ether, filtered and washed (3x) with diethyl ether to afford the compounds **2a-r**.

*(±)-2-[(1-Benzylpyridin-1-ium-4-yl)methyl]-5,6-dimethoxy-indan-1-one bromide (**2a**).* The title compound **2a** was prepared according to the general procedure C with compound **8** (48 mg, 0.17 mmol) and benzyl bromide (41 µL, 0.34 mmol) as reactants. Pale brown solid solid. (Yield 61.3 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.75 (dd, 1H, *J* = 17.1 Hz, *J* = 3.3 Hz), 3.05-3.14 (m, 2H), 3.30-3.41 (m, 2H), 3.89 (s, 3H), 3.96 (s, 3H), 6.17 (s, 2H), 6.87 (s, 1H), 7.11 (s, 1H), 7.40-7.42 (m, 3H), 7.61-7.64 (m, 2H), 7.92 (d, 2H, *J* = 6.6 Hz), 9.23 (d, 1H, *J* = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 32.2, 37.2, 47.1, 56.2, 56.6, 63.8, 104.3, 107.7, 128.3, 128.7, 129.8, 129.9, 130.3, 132.3, 144.0, 148.5, 149.9, 156.3, 161.2, 204.2. Anal. Calcd. for $C_{24}H_{24}BrNO_3$: C, 63.44; H, 5.32; N, 3.08. Found: C, 63.39; H, 5.24; N, 3.15.

*(±)-1-Benzyl-4-[(5,6-dimethoxy-1-oxo-indan-2-yl)methyl]pyridin-1-ium-3-carbonitrile bromide (**2b**).* The title compound **2b** was prepared according to the general procedure C with compound **15** (100.0 mg, 0.32 mmol) and benzyl bromide (66 µL, 0.55 mmol). Pale yellow solid. (Yield 74.2 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.81-2.85 (m, 1H), 3.23-3.25 (m, 3H), 3.42-3.48 (m, 1H), 3.8 (s, 3H), 3.87 (s, 3H), 5.87 (s, 2H), 7.09 (s, 1H), 7.13z (s, 1H), 7.46-7.48 (m, 3H), 7.60-7.62 (m, 2H), 8.41 (d, 1H, *J* = 6.5Hz), 9.38 (d, 1H, *J* = 6.5Hz), 9.98 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 31.7, 35.5, 46.2, 55.7, 56.1, 63.4, 104.1, 108.2, 113.1, 114.2, 127.6, 129.0, 129.1, 129.3, 129.6, 133.62, 146.8, 148.6, 149.4, 155.8, 163.8, 203.6. HRMS (ESI$^+$) *m/z* 399.1707 $[M]^+$, calcd for $C_{25}H_{23}N_2O_3$ 399.1709. Anal. Calcd. for $C_{25}H_{23}BrN_2O_3$: C, 62.64; H, 4.84; N, 5.84. Found: C, 62.71; H, 4.85; N, 5.87.

*(±)-2-[(3-Acetyl-1-benzyl-pyridin-1-ium-4-yl)methyl]-5,6-dimethoxy-indan-1-one bromide (**2c**).* The title compound **2c** was prepared according to the general procedure C with compound

10a (46 mg, 0.14 mmol) and benzyl bromide (34 μL, 0.28 mmol) as reactants. Pale brown solid. (Yield 62.3 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.75 (dd, 1H, *J* = 3.9Hz), 2.98 (s, 3H), 3.00-3.07 (m, 1H), 3.29-3.35 (m, 1H), 3.37-3.48 (m, 2H), 3.87 (s, 3H), 3.95 (s, 3H), 6.46 (s, 2H), 6.84 (s, 1H), 7.06 (s, 1H), 7.38-7.40 (m, 3H), 7.72-7.75 (m, 2H), 7.96 (d, 1H, *J* = 6.5 Hz), 9.17 (d, 1H, *J* = 6.5 Hz), 10.4 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.4, 32.9, 35.5, 47.4, 56.3, 56.5, 63.6, 104.5, 107.6, 128.4, 129.9, 130.0, 130.3, 131.0, 132.8, 136.3, 144.4, 146.0, 148.4, 149.9, 156.3, 161.4, 196.7, 204.6. HRMS (ESI$^+$) *m/z* 416.1865 $[M]^+$, calcd for $C_{26}H_{26}NO_4$ 416.1862. Anal. Calcd. for $C_{26}H_{26}BrNO_4$: C, 62.91; H, 5.28; N, 2.82. Found: C, 62.65; H. 5.26; N, 2.88.

*(±)-2-[[3-Acetyl-1-(o-tolylmethyl)pyridin-1-ium-4-yl]methyl]-5,6-dimethoxy-indan-1-one bromide (**2d**).* The title compound **2d** was prepared according to the general procedure C with compound **10a** (130.1 mg, 0.4 mmol) and 2-methylbenzyl bromide (90 μL, 0.68 mmol) as reactants. Pale brown solid. (Yield 130 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.36 (s, 3H), 2.76 (dd, 1H, *J* = 16.8 Hz, *J* = 3.6 Hz), 2.95 (s, 3H), 3.00-3.10 (m, 1H), 3.36 (dd, 1H, *J* = 16.8, *J* = 7.4Hz), 3.42 (d, 1H, *J* = 7.4Hz), 3.88 (s, 3H), 3.95 (s, 3H), 6.45 (s, 2H), 6.85 (s, 1H), 7.05 (s, 1H), 7.22-7.36 (m, 3H), 7.59 (d, 1H, *J* = 7.5 Hz), 7.95 (d, 1H, *J* = 6.6 Hz), 8.81 (d, 1H, *J* = 6.6 Hz), 10.20 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.9, 31.3, 32.9, 35.6, 47.4, 56.2, 56.5, 62.5, 104.3, 107.5, 127.4, 128.3, 130.5, 130.7, 130.8, 131.5, 131.7, 135.8, 138.3, 143.9, 146.2, 148.5, 149.9, 156.2, 161.3, 196.7, 204.8. HRMS (ESI$^+$) *m/z* 430.2021 $[M]^+$, calcd for $C_{27}H_{28}NO_4$ 430.2018. Anal. Calcd. for $C_{27}H_{28}BrNO_4$: C, 63.53; H, 5.53; N, 2.74, Found: C, 63.81; H, 5.82; N, 2.85.

*(±)-2-[[3-Acetyl-1-(m-tolylmethyl)pyridin-1-ium-4-yl]methyl]-5,6-dimethoxy-indan-1-one bromide (**2e**).* The title compound **2e** was prepared according to the general procedure C with

compound **10a** (80 mg, 0.246 mmol) and 3-methylbenzyl bromide (40 μL, 0.29 mmol) as reactants. Pale brown solid. (Yield 107.3 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.31 (s, 3H), 2.74 (dd, 1H, *J* = 17.1 Hz, *J* = 3.8 Hz), 2.97 (s, 3H), 2.99-3.06 (m, 1H), 3.27-3.36 (m, 2H), 3.40-3.48 (m, 1H), 3.87 (s, 3H), 3.94 (s, 3H), 6.39 (s, 2H), 6.83 (s, 1H), 7.05 (s, 1H), 7.16-7.28 (m, 2H), 7.49-7.53 (m, 2H), 7.98 (d, 1H, *J* = 6.3 Hz), 9.22 (d, 1H, *J* = 6.3 Hz), 10.34 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 21.4, 31.4, 32.8, 35.4, 47.3, 56.2, 56.5, 63.5, 104.4, 107.5, 126.9, 128.4, 129.7, 130.4, 130.8, 130.9, 132.8, 136.2, 139.8, 144.7, 145.8, 148.4, 149.9, 156.2, 161.1, 196.7, 204.6. HRMS (ESI$^+$) *m/z* 430.2014 $[M]^+$, calcd for $C_{27}H_{28}NO_4$ 430.2018. Anal. Calcd. for $C_{27}H_{28}BrNO_4$: C, 63.53; H, 5.53; N, 2.74. Found: C, 63.67; H, 5.82; N, 2.85.

*(±)-2-[[3-Acetyl-1-[(2-chlorophenyl)methyl]pyridin-1-ium-4-yl]methyl]-5,6-dimethoxy-indan-1-one bromide (**2f**).* The title compound **2f** was prepared according to the general procedure C with compound **10a** (80 mg, 0.246 mmol) and 2-chlorobenzyl bromide (66 μL, 0.49 mmol) as reactants. Pale brown solid. (Yield 107.2 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.75 (dd, 1H, *J* = 16.8 Hz, *J* = 3.9 Hz), 2.97 (s, 3H), 3.00-3.09 (m, 1H), 3.35 (dd, 1H, *J* =16.8 Hz, *J* = 7.35), 3.42-3.5 (m, 2H), 3.88 (s, 3H), 3.95 (s, 3H), 6.50 (d, 2H, *J* = 2.7 Hz), 6.85 (s, 1H), 7.07 (s, 1H), 7.39-7.43 (m, 3H), 7.99 (d, 1H, *J* = 6.6 Hz), 8.28-8.31 (m, 1H), 9.01 (d, 1H, *J* = 5.7 Hz), 10.20 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.1, 32.8, 35.5, 47.3, 56.2, 56.4, 61.4, 104.3, 107.5, 128.3, 128.5, 130.4, 130.6, 132.1, 133.7, 135.0, 135.8, 144.6, 146.2, 148.4, 149.8, 156.2, 161.6, 196.5, 204.6. HRMS (ESI$^+$) *m/z* 450.1465 $[M]^+$, calcd for $C_{26}H_{25}ClNO_4$ 450.1472. Anal. Calcd. for $C_{26}H_{25}BrClNO_4$: C, 58.83; H, 4.75; N, 2.64. Found: C, 58.52; H, 4.77; N, 2.43.

*(±)-2-[[3-Acetyl-1-[(3-chlorophenyl)methyl]pyridin-1-ium-4-yl]methyl]-5,6-dimethoxy-indan-1-one bromide (**2g**).* The title compound **2g** was prepared according to the general procedure C with compound **10a** (95.1 mg, 0.29 mmol) and 3-chlorobenzyl bromide (65 μL, 0.49 mmol) as

reactants. Pale brown solid. (Yield 108 mg, 70%). ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.73-2.80 (m, 1H), 2.75 (s, 3H), 3.13-3.18 (m, 3H), 3.52-3.61 (m, 1H), 3.79 (s, 3H), 3.86 (s, 3H), 5.93 (s, 2H), 7.06 (s, 1H), 7.09 (s, 1H), 7.50-7.60 (m, 2H), 7.61-7.64 (m, 1H), 7.81 (s, 1H), 8.28 (d, 1H, J = 6.3 Hz), 9.23 (dd, 1H, J = 6.3 Hz, J = 1.2 Hz), 9.79 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 30.5, 31.8, 34.2, 46.5, 55.7, 56.1, 61.9, 104.0, 108.2, 127.8, 127.9, 129.0, 129.5, 130.4, 131.1, 133.7, 136.3, 136.7, 144.9, 145.6, 148.6, 149.3, 155.6, 160.3, 197.4, 204.2. HRMS (ESI$^+$) *m/z* 450.1472 $[M]^+$, calcd for $C_{26}H_{25}ClNO_4$ 450.1472. Anal. Calcd. for $C_{26}H_{25}BrClNO_4$: C, 58.83 H, 4.75; N, 2.64. Found: C, 58.79; H, 4.80; N, 2.62.

*(±)-6-[[3-Acetyl-1-(o-tolylmethyl)pyridin-1-ium-4-yl]methyl]-5,6-dihydrocyclopenta[f][1,3] benzodioxol-7-one bromide (**2h**).* The title compound **2h** was prepared according to the general procedure C with compound **10e** (110.1 mg, 0.35 mmol) and 2-methylbenzyl bromide (80 µL, 0.60 mmol) as reactants. Pale brow solid. (Yield 135 mg, 77%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.37 (s, 3H), 2.75 (dd, 1H, J = 16.8 Hz, J = 3.6 Hz), 2.97 (s, 3H), 3.01-3.10 (m, 1H), 3.31-3.39 (m, 1H), 3.40-3.51 (m, 2H), 6.07 (s, 2H), 6.47 (s, 2H), 6.80 (s, 1H), 6.98 (s, 1H), 7.29-7.39 (m, 2H), 7.58 (d, 1H, J = 7.2 Hz), 7.95 (d, 1H, J = 6.3 Hz), 8.77 (d, 1H, J = 6.0 Hz), 10.32 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.9, 31.3, 33.2, 35.6, 47.6, 62.6, 102.5, 105.9, 127.5, 130.2, 130.4, 130.8, 130.9, 131.5, 131.8, 135.8, 138.3, 143.7, 146.3, 148.8, 150.7, 155.1, 161.3, 196.7, 204.1. HRMS (ESI$^+$) *m/z* 414.1700 $[M]^+$, calcd for $C_{26}H_{24}NO_4$ 414.1705. Anal. Calcd. for $C_{26}H_{24}BrNO_4$: C, 63.17; H, 4.89; N, 2.83. Found: C, 63.41; H, 4.80; N, 2.62.

*(±)-2-[[3-Acetyl-1-(o-tolylmethyl)pyridin-1-ium-4-yl]methyl]-5-methoxy-indan-1-one bromide (**2i**).* The title compound **2i** was prepared according to the general procedure C with compound **10c** (111 mg, 0.37 mmol) and 2-methylbenzyl bromide (84 µL, 0.63 mmol) as reactants. Pale brown solid. (Yield 145.2 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.36 (s, 3H), 2.80 (dd, 1H,

J = 17.1 Hz, *J* = 3.9 Hz), 2.95 (s, 3H), 3.03-3.06 (m, 1H), 3.35-3.48 (m, 3H), 3.86 (s, 3H), 6.46 (s, 2H), 6.85-6.89 (m, 2H), 7.21-7.36 (m, 3H), 7.55-7.61 (m, 2H), 7.99 (d, 1H, *J* = 6.6 Hz), 8.88 (d, 1H, *J* = 6.6 Hz), 10.26 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.9, 31.2, 33.2, 35.5, 47.3, 55.8, 62.3, 109.6, 116.1, 125.8, 127.3, 128.8, 130.5, 130.6, 130.9, 131.3, 131.6, 135.8, 138.2, 144.2, 146.1, 156.0, 161.2, 165.9, 196.7, 204.3. HRMS (ESI^+) *m/z* 400.1908 $[M]^+$, calcd for $C_{26}H_{26}NO_3$ 400.1913. Anal. Calcd. for $C_{26}H_{26}BrNO_3$: C, 65.01; H, 5.46; N, 2.92. Found: C, 64.90; H, 5.37; N, 2.87.

*(±)-2-[[3-Acetyl-1-(o-tolylmethyl)pyridin-1-ium-4-yl]methyl]-5,6-dimethyl-indan-1-one bromide (**2j**).* The title compound **2j** was prepared according to the general procedure C with compound **10d** (41.1 mg, 0.14 mmol) and 2-methylbenzyl bromide (32 μL, 0.24 mmol) as reactants. Pale yellow solid. (Yield 66 mg, 97%). ^{1}H NMR (300 MHz, CD_3OD, δ): 2.32 (s, 3H), 2.37 (s, 6H), 2.72 (s, 3H), 2.87 (dd, 1H, *J* = 16.8 Hz, *J* = 3.3 Hz), 3.16-3.23 (m, 1H), 3.32-3.41 (m, 2H), 3.61 (dd, 1H, *J* = 13.5 Hz, *J* = 7.8 Hz), 5.96 (s, 2H), 7.28-7.43 (m, 6H), 8.17 (d, 1H, *J* = 6.1 Hz), 8.78 (d, 1H, *J* = 6.1 Hz), 9.46 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD, δ): 19.3, 19.8, 20.8, 30.0, 33.5, 36.1, 63.4, 124.9, 128.3, 128.5, 131.0, 131.4, 132.2, 132.3, 132.8, 135.1, 138.1, 138.3, 138.9, 145.7, 146.6, 147.5, 153.2, 162.8, 197.7, 208.3. HRMS (ESI^+) *m/z* 398.2120 $[M]^+$, calcd for $C_{27}H_{28}NO_2$ 398.2120. Anal. Calcd. for $C_{27}H_{28}BrNO_2$: C, 67.78; H, 5.90; N, 2.93. Found: C, 68.11; H, 5.53; N, 2.85.

*(±)-2-[[3-Acetyl-1-(o-tolylmethyl)pyridin-1-ium-4-yl]methyl]-5-methyl-indan-1-one bromide (**2k**).* The title compound **2k** was prepared according to the general procedure C with compound **10b** (55.8 mg, 0.2 mmol) and 2-methylbenzyl bromide (45 μL, 0.34 mmol) as reactants. Pale brown solid. (Yield 78.9 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.37 (s, 3H), 2.43 (s, 3H), 2.83 (dd, 1H, *J* = 17.1 Hz, *J* = 4.2 Hz), 2.98 (s, 3H), 3.04-3.10 (m, 1H), 3.35-3.50 (m, 3H), 6.44

(s, 2H), 7.17-7.39 (m, 5H), 7.54-7.58 (m, 2H), 7.95 (d, 1H, *J* = 6.0 Hz), 8.73 (d, 1H, *J* = 6.0 Hz), 10.28 (s, 1H). HRMS (ESI^+) *m/z* 384.1963 $[M]^+$, calcd for $C_{26}H_{26}NO_2$ 384.1964. Anal. Calcd. for $C_{26}H_{26}BrNO_2$: C, 67.24; H, 5.64; N, 3.02. Found: C, 67.56; H, 5.80; N, 2.94.

*(±)-2-[[3-Acetyl-1-(o-tolylmethyl)pyridin-1-ium-4-yl]methyl]-5-chloro-indan-1-one bromide (**2l**).* The title compound **2l** was prepared according to the procedure C with compound **10f** (90 mg, 0.3 mmol) and 2-methylbenzyl bromide (68 µL, 0.51 mmol) as reactants. White solid. (Yield 119.2 mg, 82%). ^1H NMR (300 MHz, CD_3OD, δ): 2.30-2.38 (m, 1H), 2.38 (s, 3H), 2.73 (s, 3H), 2.97 (dd, 1H, *J* = 17.1 Hz, *J* = 3.9 Hz), 3.22-3.48 (m, 2H), 3.64 (dd, 1H, *J* = 13.5 Hz, *J* = 7.8 Hz), 5.98 (s, 2H), 7.29-7.45 (m, 5H), 7.59 (s, 1H), 7.65 (d, 1H, *J* = 8.4 Hz), 8.19 (d, 1H, *J* = 6.6 Hz), 8.79 (d, 1H, *J* = 6.6 Hz), 9.48 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD, δ): 19.2, 29.9, 33.7, 35.9, 63.4, 126.1, 128.0, 128.3, 129.5, 131.0, 131.4, 132.2, 132.4, 132.6, 135.7, 138.3, 138.9, 142.8, 145.8, 146.7, 156.5, 162.5, 197.6, 206.9. HRMS (ESI^+) *m/z* 404.1410 $[M]^+$, calcd for $C_{25}H_{23}ClNO_2$ 404.1417. Anal. Calcd. for $C_{25}H_{23}BrClNO_2$: C, 61.94; H, 4.78; N, 2.89. Found: C, 61.85; H, 4.66; N, 2.67.

*(±)-1-Benzyl-4-[(5,6-dimethoxy-1-oxo-indan-2-yl)methyl]-N-methyl-pyridin-1-ium-3-carboxamide bromide (**2m**).* The title compound **2m** was prepared according to the general procedure C with compound **13** (100.0 mg, 0.29 mmol) and benzyl bromide (59 µL, 0.5 mmol) as reactants. Pale yellow solid. (Yield 140.1 mg, 93%). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.78 (dd, 1H, *J* = 16.8 Hz, *J* = 3.6 Hz), 2.95 (d, 3H, *J* = 4.8 Hz), 3.06-3.14 (m, 1H), 3.25 (dd, 1H, *J* = 17.1 Hz, *J* = 7.8 Hz), 3.33-3.46 (m, 2H), 3.85 (s, 3H), 3.92 (s, 3H), 5.94 (s, 2H), 6.79 (s, 1H), 7.04 (s, 1H), 7.39-7.41 (m, 3H), 7.54-7.57 (m, 2H), 7.91 (d, 1H, *J* = 6.3 Hz), 8.87 (d, 1H, *J* = 6.3 Hz), 9.17-9.20 (m, 1H), 9.35 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 26.8, 32.5, 34.8, 47.6, 56.2, 56.4, 64.2, 104.4, 107.5, 128.4, 129.7, 129.8, 129.9, 130.5, 131.8, 135.3, 142.2, 144.2, 148.6,

149.8, 156.2, 161.2, 163.6, 204.6. HRMS (ESI^+) *m/z* 431.1968 $[M]^+$, calcd for $C_{26}H_{27}N_2O_4$ 431.1971. Anal. Calcd. for $C_{26}H_{27}BrN_2O_4$: C, 61.06; H, 5.32; N, 5.48. Found: C, 61.19; H, 5.24; N, 5.30.

*(±)-4-[(5,6-Dimethoxy-1-oxo-indan-2-yl)methyl]-N-methyl-1-(o-tolylmethyl)pyridin-1-ium-3-carboxamide bromide (**2n**).* The title compound **2n** was prepared according to the general procedure C with compound **13** (51.1 mg, 0.15 mmol) and 2-methylbenzyl bromide (40 µL, 0.3 mmol) as reactants. Yellow solid. (Yield 47.5 mg, 71%). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.29 (s, 3H), 2.79 (dd, 1H, *J* = 16.8 Hz, *J* = 3.6 Hz), 2.96 (d, 3H, *J* = 4.5 Hz), 3.11-3.17 (m, 1H), 3.27 (dd, 1H, *J* = 17.1 Hz, *J* = 7.6 Hz), 3.34-3.49 (m, 2H), 3.86 (s, 3H), 3.93 (s, 3H), 5.96 (s, 2H), 6.82 (s, 1H), 7.07 (s, 1H), 7.24-7.39 (m, 3H), 7.51 (d, 1H, *J* = 7.2 Hz), 7.89 (d, 1H, *J* = 6.3 Hz), 8.54 (d, 1H, *J* = 6.3 Hz), 9.22 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.7, 26.8, 32.5, 34.8, 47.7, 56.2, 56.5, 62.7, 104.4, 107.6, 127.6, 128.4, 129.4, 129.5, 130.9, 131.6, 131.8, 135.4, 137.9, 141.7, 144.2, 148.7, 149.8, 156.2, 161.1, 163.7, 204.7. HRMS (ESI^+) *m/z* 445.2140 $[M]^+$, calcd for $C_{27}H_{29}N_2O_4$ 445.2127. Anal. Calcd. for $C_{27}H_{29}BrN_2O_4$: C, 61.72; H, 5.56; N, 5.33. Found: C, 61.40; H, 5.47; N, 4.96.

*(±)-Methyl 1-benzyl-4-[(5,6-dimethoxy-1-oxo-indan-2-yl)methyl]pyridin-1-ium-3-carboxylate bromide (**2o**).* The title compound **2o** was prepared according to the general procedure C with compound **12** (60.1 mg, 0.176 mmol) and benzyl bromide (42µL, 0.35 mmol) as reactants. Pale yellow solid. (Yield 89.5 mg, 99%). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.75 (dd, 1H, *J* = 16.8 Hz, *J* = 3.6 Hz), 3.01-3.10 (m, 1H), 3.28 (dd, 1H, *J* = 17.1Hz, *J* = 7.8Hz), 3.37 (dd, 1H, *J* = 13.8Hz, *J* = 8.1Hz), 3.68 (dd, 1H, *J* = 13.8 Hz, *J* = 7.2 Hz), 3.84 (s, 3H), 3.91 (s, 3H), 3.92 (s, 3H), 6.38 (s, 2H), 6.82 (s, 1H), 7.04 (s, 1H), 7.27-7.36 (m, 3H), 7.68-7.71 (m, 2H), 8.12 (d, 1H, *J* = 6.2 Hz), 8.67 (s, 1H), 9.78 (d, 1H, *J* = 6.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 32.6, 35.7, 47.3, 53.7,

56.1, 56.4, 63.9, 104.3, 107.5, 128.2, 128.4, 128.8, 129.0, 129.6, 129.7, 130.0, 130.9, 132.6, 146.0, 146.2, 148.3, 149.7, 156.0, 162.3, 162.6, 204.3. HRMS (ESI^+) *m/z* 432.1806 $[M]^+$, calcd for $C_{26}H_{26}NO_5$ 432.1811. Anal. Calcd. for $C_{26}H_{26}BrNO_5$: C, 60.95; H, 5.11; N, 2.73. Found: C, 60.71; H, 5.01; N, 2.61.

*(±)-Methyl 4-[(5,6-dimethoxy-1-oxo-indan-2-yl)methyl]-1-(o-tolylmethyl)pyridin-1-ium-3-carboxylate bromide (**2p**).* The title compound **2p** was prepared according to the general procedure C with compound **12** (50.1 mg, 0.146 mmol) and 2-methylbenzyl bromide (33 µL, 0.25 mmol) as reactants. Pale yellow solid. (Yield 75.1 mg, 97%). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.36 (s, 3H), 2.82 (dd, 1H, *J* = 17.1 Hz, *J* = 3.6 Hz), 3.09-3.14 (m, 1H), 3.38 (dd, 1H, *J* = 7.8Hz, *J* = 16.5Hz), 3.44-3.53 (m, 2H), 3.73 (dd, 1H, *J* = 13.5 Hz, *J* = 7.8 Hz), 3.89 (s, 3H), 3.97 (s, 6H), 6.33 (s, 2H), 6.88 (s, 1H), 7.09 (s, 1H), 7.29-7.42 (m, 3H), 7.54 (d, 1H, *J* = 8.4 Hz), 8.09 (d, 1H, *J* = 6.3 Hz), 9.30 (s, 2H), 9.55 (d, 1H, *J* = 6.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.9, 32.9, 35.9, 47.4, 53.9, 56.2, 56.5, 63.1, 104.4, 107.6, 127.5, 128.4, 129.5, 130.2, 130.7, 131.0, 131.5, 131.7, 138.2, 145.7, 146.3, 148.6, 149.9, 156.2, 162.5, 162.8, 204.5. HRMS (ESI^+) *m/z* 446.1979 $[M]^+$, calcd for $C_{27}H_{28}NO_5$ 446.1967. Anal. Calcd. for $C_{27}H_{28}BrNO_5$: C, 61.60; H, 5.36; N, 2.66. Found: C, 61.51; H, 5.36; N, 2.43.

*(±)-4-[(5,6-Dimethoxy-1-oxo-indan-2-yl)methyl]-1-(o-tolylmethyl)pyridin-1-ium-3-carboxamide bromide (**2q**).* The title compound **2q** was prepared according to the general procedure C with compound **14** (100.2 mg, 0.31 mmol) and 2-methylbenzyl bromide (70 µL, 0.52 mmol) as reactants. Yellow solid. (Yield 150 mg, 95%). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.32 (s, 3H), 2.80 (dd, 1H, *J* = 17.1 Hz, *J* = 3.9 Hz), 3.14-3.20 (m, 1H), 3.32 (dd, 1H, *J* = 17.1 Hz, *J* = 7.8 Hz), 3.40-3.53 (m, 2H), 3.88 (s, 3H), 3.95 (s, 3H), 5.99 (s, 2H), 6.15 (s, 1H), 6.86 (s, 1H), 7.09 (s, 1H), 7.28-7.43 (m, 3H), 7.53 (d, 1H, *J* = 7.5 Hz), 7.90 (d, 1H, *J* = 6.6 Hz), 8.31 (d,

1H, *J* = 6.0 Hz), 9.26 (s, 1H), 9.47 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.7, 32.6, 34.9, 47.8, 56.3, 56.5, 62.9, 104.4, 107.7, 127.7, 128.4, 129.2, 129.8, 131.1, 131.8, 131.9, 134.6, 138.1, 141.3, 144.7, 148.8, 149.9, 156.3, 161.4, 164.9, 204.9. HRMS (ESI^+) *m/z* 431.1974 $[M]^+$, calcd for $C_{26}H_{27}N_2O_4$ 431.1971;. Anal. Calcd. for $C_{26}H_{27}BrN_2O_4$: C, 61.06; H, 5.32; N, 5.48. Found: C, 61.49; H, 5.39; N, 5.45.

*(±)-1-[(3-Chlorophenyl)methyl]-4-[(5,6-dimethoxy-1-oxo-indan-2-yl)methyl]pyridin-1-ium-3-carboxamide bromide (**2r**).* The title compound **2r** was prepared according to the general procedure C with compound **13** (1.40 g, 4.29 mmol) and 3-chlorobenzyl bromide (1.12 mL, 8.58 mmol) as reactants. Yellow solid. (Yield 2.24 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.74-2.80 (m, 1H), 3.05-3.20 (m, 1H), 3.26 (dd, 1H, *J* = 16.8 Hz, *J* = 7.5 Hz), 3.33-3.38 (m, 1H), 3.49 (dd, 1H, *J* = 14.2 Hz, *J* = 6.6Hz), 3.86 (s, 3H), 3.92 (s, 3H), 6.07 (s, 2H), 6.70 (s, 1H), 6.82 (s, 1H), 7.04 (s, 1H), 7.36 (br s, 2H), 7.61 (br s, 2H), 7.99 (d, 1H, *J* = 6.2 Hz), 8.51 (d, 1H, *J* = 6 Hz), 8.94 (s, 1H), 9.63 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 31.6, 33.9, 46.6, 55.7, 56.1, 61.8, 104.1, 108.2, 127.8, 127.9, 129.2, 129.5, 131.1, 133.7, 136.3, 136.7, 143.0, 144.1, 148.5, 149.3, 155.6, 158.6, 164.8, 203.9. HRMS (ESI^+) *m/z* 451.1423 $[M]^+$, calcd for $C_{25}H_{24}N_2O_4Cl$ 451.1425. Anal. Calcd. for $C_{25}H_{24}BrClN_2O_4$: C, 56.46; H, 4.55; N, 5.27. Found: C, 56.70; H, 4.47; N, 4.97.

General Procedure D for Synthesis of Compounds 6,7a-f. Compounds **6,7a-f** were prepared using the method described by Li et al.[64] To a solution of 1-indanone derivatives (2 mmol) and 4-pyridinecarboxaldehyde derivatives (2 mmol) in toluene (30 mL) was added *p*-toluenesulfonic acid (2.4 mmol). After heated at reflux using a Dean-Stark apparatus for 4h, the mixture was cooled to room temperature and the solvent was removed in vacuum, then 5% sodium bicarbonate aqueous solution was added until reach pH = 8. After extraction with dichloromethane (4x), the organic layer was dried over magnesium sulfate and concentrated to

dryness. The residue was taken up with EtOAc and the solid was filtered, rinsed with EtOAc to afford compounds **6,7a-f**.

*(2E)-5,6-Dimethoxy-2-(4-pyridylmethylene)indan-1-one (**6**).* The title compound **6** was prepared according to the general procedure D with 5,6-dimethoxy-1-indanone (0.38 g, 2 mmol) and 4-pyridinecarboxaldehyde **4** (0.21 g, 2 mmol) as reactants. Yellow solid. (Yield 0.43 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.94 (s, 3H), 3.97 (d, 2H, *J* = 1.2 Hz), 4.00 (s, 3H), 6.97 (s, 1H), 7.32 (s, 1H), 7.44-7.46 (m, 3H), 8.69 (d, 2H, *J* = 5.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.5, 56.3, 56.5, 105.2, 107.2, 123.9, 129.1, 130.8, 139.9, 142.7, 144.8, 149.8, 150.4, 156.1, 192.1. HRMS (ESI^+) *m/z* 282.1122 $[M+H]^+$, calcd for $C_{17}H_{16}NO_3$ 282.1130.

*(2E)-2-[(3-Bromo-4-pyridyl)methylene]-5,6-dimethoxy-indan-1-one (**7a**).* The title compound **7a** was prepared according to the general procedure D with 5,6-dimethoxy-1-indanone **3a** (1.92 g, 10 mmol) and 3-bromo-4-pyridinecarboxaldehyde **5** (1.87 g, 10 mmol) as reactants. Pale yellow solid. (Yield 2.34 g, 65%). Rf = 0.16 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.87-3.88 (m, 2H), 3.95 (s, 3H), 3.99 (s, 3H), 6.93 (s, 1H), 7.34 (s, 1H), 7.49 (d, 1H, *J* = 5.1 Hz), 7.72 (s, 1H), 8.57 (d, 1H, *J* = 5.1 Hz), 8.81 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.4, 56.3, 56.5, 105.2, 107.2, 123.5, 123.6, 127.9, 130.8, 141.3, 143.1, 145.0, 148.3, 150.0, 153.0, 156.1, 191.7. HRMS (ESI^+) *m/z* 360.0231 $[M+H]^+$, calcd for $C_{17}H_{15}BrNO_3$ 360.0235.

*(2E)-2-[(3-Bromo-4-pyridyl)methylene]-5-methyl-indan-1-one (**7b**).* The title compound **7b** was prepared according to the general procedure D with 5-methyl-1-indanone **3b** (0.73 g, 5 mmol) and 3-bromo-4-pyridinecarboxaldehyde **5** (0.93 g, 5 mmol) as reactants. White solid. (Yield 1.24 g, 79%). Rf = 0.29 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.46 (s, 3H), 3.90 (s, 2H), 7.23-7.30 (m, 2H), 7.50 (d, 1H, *J* = 5,1 Hz), 7.76-7.81 (m, 2H), 8.58

(d, 1H, *J* = 5.1 Hz), 8.80 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 22.3, 31.4, 123.4, 123.6, 124.5, 126.5, 128.6, 129.2, 135.1, 140.9, 142.6, 146.8, 148.8, 149.8, 152.8, 192.5. HRMS (ESI^+) *m/z* 314.0185 $[M+H]^+$, calcd for $C_{16}H_{13}BrNO$ 314.0181.

*(2E)-2-[(3-Bromo-4-pyridyl)methylene]-5-methoxy-indan-1-one (**7c**).* The title compound **7c** was prepared according to the general procedure D with 5-methoxy-indanone **3c** (324.4 mg, 2 mmol) and 3-bromo-4-pyridinecarboxaldehyde **5** (0.37 g, 2 mmol) as reactants. Yellow solid. (Yield 0.33 g, 50%). Rf = 0.25 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.91 (br s, 5H), 6.96-7.00 (m, 2H), 7.49 (d, 1H, *J* = 5,1 Hz), 7.74 (t, 1H, *J* = 2.4 Hz), 7.87 (d, 1H, *J* = 8.4 Hz), 8.58 (d, 1H, *J* = 5.1 Hz), 8.82 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.7, 55.8, 109.8, 115.8, 123.5, 126.7, 128.2, 131.1, 141.2, 143.1, 148.3, 152.3, 153.0, 165.8, 191.3. HRMS (ESI^+) *m/z* 330.0129 $[M+H]^+$, calcd for $C_{16}H_{13}BrNO_2$ 330.0130.

*(2E)-2-[(3-Bromo-4-pyridyl)methylene]-5,6-dimethyl-indan-1-one (**7d**).* The title compound **7d** was prepared according to the general procedure D with 5,6-dimethyl-1-indanone (0.40 g, 2.5 mmol) and 3-bromo-4-pyridinecarboxaldehyde **5** (0.45 g, 2.5 mmol) as reactants. Yellow solid. (Yield 0.48 g, 58%). Rf = 0.36 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.31 (s, 3H), 2.35 (s, 3H), 3.84 (s, 2H), 7.26 (s, 1H), 7.50 (s, 1H), 7.63 (s, 1H), 7.72 (s, 1H), 8.57 (br s, 1H), 8.80 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.9, 21.0, 31.2, 125.2, 127.0, 128.5, 135.8, 137.1, 141.3, 143.0, 146.0, 147.6, 148.3, 152.9, 192.8. HRMS (ESI^+) *m/z* 328.0336 $[M+H]^+$, calcd for $C_{17}H_{15}BrNO$ 328.0337.

*(6E)-6-[(3-Bromo-4-pyridyl)methylene]-5H-cyclopenta[f][1,3]benzodioxol-7-one (**7e**).* The title compound **7e** was prepared according to the general procedure D with 5,6-methylenedioxy-1-indanone (0.50 g, 2.87 mmol) and 3-bromo-4-pyridinecarboxaldehyde **5** (0.55 g, 3 mmol) as reactants. Yellow solid. (Yield 0.35 g, 35%). Rf = 0.18 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR

(300 MHz, $CDCl_3$, δ): 3.85 (d, 2H, *J* = 1,8 Hz), 6.11 (s, 2H), 6.89 (s, 1H), 7.28 (s, 1H), 7.49 (d, 1H, *J* = 5,1 Hz), 7.71 (t, 1H, *J* = 2.1 Hz), 8.59 (br s, 1Hz), 8.82 (s, 1H). HRMS (ESI^+) *m/z* 343.9910 $[M+H]^+$, calcd for $C_{16}H_{11}BrNO_3$ 343.9922.

*(2E)-2-[(3-Bromo-4-pyridyl)methylene]-5-chloro-indan-1-one (**7f**).* The title compound **7f** was prepared according to the general procedure D with 5-chloro-1-indanone (0.83 g, 5 mmol) and 3-bromo-4-pyridinecarboxaldehyde **5** (0.93 g, 5 mmol) as reactants. White solid. (Yield 0.44 g, 26%). Rf = 0.51 (EtOAc/Petroleum ether = 3:2). ^1H NMR (300 MHz, $CDCl_3$, δ): 3.95 (s, 2H), 7.43-7.52 (m, 3H), 7.82 (d, 1H, *J* = 2.1 Hz), 7.87 (d, 1H, *J* = 5,1 Hz), 8.61 (d, 1H, *J* = 4.8 Hz), 8.83 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.4, 123.4, 123.60, 126.0, 126.5, 129.0, 130.0, 136.0, 139.8, 141.8, 142.6, 148.4, 150.8, 153.0, 191.7. HRMS (ESI^+) *m/z* 333.9639 $[M+H]^+$, calcd for $C_{15}H_{10}BrClNO$ 333.9634.

General procedure E for synthesis of compounds 8,9a-f. Pt/C (5 wt% loading, 10% w/w) was added to a solution of the corresponding compound **6,7a-f** (4 mmol) in EtOH (40 mL) at room temperature. Then, the resulting suspension was stirred for 3 hours under H_2 atmosphere (1 atm) then filtered over Celite®, rinsed with dichloromethane and the filtrate was concentrated to dryness to afford compounds **8,9a-f** which were used without any purifications.

*5,6-Dimethoxy-2-(4-pyridylmethyl)indan-1-one (**8**).* The title compound **8** was prepared according to the general procedure E with compound **6** (210 mg, 0.74 mmol) and Pt/C (5 wt% loading, 22 mg) as reactants. White solid. (Yield 206 mg, 97%). Rf = 0.38 (EtOAc/Petroleum ether = 3:2). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.65-2.73 (m, 2H), 2.98-3.02 (m, 1H), 3.11 (dd, 1H, *J* = 16.8 Hz, *J* = 7.5 Hz), 3.35 (dd, 1H, *J* = 14.1 Hz, *J* = 4.2 Hz), 3.91 (s, 3H), 3.94 (s, 3H), 6.81 (s, 1H), 7.17-7.19 (m, 3H), 8.52 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.7, 36.4, 47.9, 56.1, 56.2, 104.3, 107.3, 124.3, 128.9, 148.6, 148.8, 149.6, 149.8, 155.8, 205.5.

*2-[(3-Bromo-4-pyridyl)methyl]-5,6-dimethoxy-indan-1-one (**9a**).* The title compound **9a** was prepared according to the general procedure E with compound **7a** (2.0 g, 5.5 mmol) and Pt/C (5 wt% loading, 200 mg) as reactants. White solid. (Yield 1.60 g, 79%). Rf = 0.25 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.74-2.87 (m, 2H), 3.06-3.17 (m, 2H), 3.13-3.17 (m, 1H), 3.92 (s, 3H), 3.95 (s, 3H), 6.83 (s, 1H), 7.20 (s, 1H), 7.22 (d, 1H, *J* = 5.1 Hz), 8.42 (d, 1H, *J* = 4.8 Hz), 8.70 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.8, 36.3, 46.6, 56.1, 56.2, 104.3, 107.3, 123.4, 125.4, 128.8, 148.2, 148.4, 149.6, 152.0, 155.7, 205.0. HRMS (ESI^+) *m/z* 362.0393 $[M+H]^+$, calcd for $C_{17}H_{17}BrNO_3$ 362.0392.

*2-[(3-Bromo-4-pyridyl)methyl]-5-methyl-indan-1-one (**9b**).* The title compound **9b** was prepared according to the general procedure E with compound **7b** (1.24 g, 4 mmol) and Pt/C (5 wt% loading, 125 mg) as reactants. White solid. (Yield 0.75 g, 59%). Rf = 0.4 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.41 (s, 3H), 2.75-2.85 (m, 2H), 3.04-3.20 (m, 2H), 3.42-3.48 (m, 1H), 7.17-7.22 (m, 3H), 8.42 (d, 2H, *J* = 5.1 Hz), 8.68 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 21.6, 31.5, 35.7, 46.0, 122.9, 123.3, 125.0, 126.4, 128.4, 133.3, 145.8, 147.7, 147.8, 151.4, 153.0, 205.2.

*2-[(3-Bromo-4-pyridyl)methyl]-5-methoxy-indan-1-one (**9c**).* The title compound **9c** was prepared according to the general procedure E with compound **7c** (0.25 g, 0.757 mmol) and Pt/C (5 wt% loading, 25 mg) as reactants. White solid. (Yield 0.251 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.72-2.82 (m, 2H), 3.03-3.16 (m, 2H), 3.40 (dd, 1H, *J* = 14.4 Hz, *J* = 4.5 Hz), 3.82 (s, 3H), 6.80 (s, 1H), 6.86 (dd, 1H, *J* = 8.4 Hz, *J* = 2.1 Hz), 7.19 (d, 1H, *J* = 5.1 Hz), 7.65 (d, 1H, *J* = 8.4 Hz), 8.38 (d, 1H, *J* = 5.1 Hz), 8.64 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 32.1, 36.3, 46.6, 55.7, 109.6, 115.7, 123.4, 125.5, 125.8, 129.3, 148.2, 148.3, 152.0, 156.0, 165.6, 204.5.

*2-[(3-Bromo-4-pyridyl)methyl]-5,6-dimethyl-indan-1-one (**9d**).* The title compound **9d** was prepared according to the general procedure E with compound **7d** (0.44 g, 1.35 mmol) and Pt/C (5 wt% loading, 44 mg) as reactants. White solid. (Yield 0.39 g, 89%). Rf = 0.32 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.29 (s, 3H), 2.32 (s, 3H), 2.72-2.84 (m, 2H), 3.02-3.16 (m, 2H), 3.44 (dd, 1H, *J* = 14.1 Hz, *J* = 7.2 Hz), 7.17 (s, 1H), 7.21 (d, 1H, *J* = 4.8 Hz), 7.53 (s, 1H), 8.41 (d, 1H, *J* = 4.8 Hz), 8.67 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.8, 20.8, 31.6, 36.2, 46.6, 123.5, 124.4, 125.5, 127.2, 134.2, 136.6, 145.5, 148.2, 148.3, 151.2, 152.0, 206.2. HRMS (ESI^+) *m/z* 330.0506 $[M+H]^+$, calcd for $C_{17}H_{17}BrNO$ 330.0494.

*6-[(3-Bromo-4-pyridyl)methyl]-5,6-dihydrocyclopenta[f][1,3]benzodioxol-7-one (**9e**).* The title compound **9e** was prepared according to the general procedure E with compound **7e** (0.25 g, 0.726 mmol) and Pt/C (5 wt% loading, 25 mg) as reactants. White solid. (Yield 0.25 g, 99%). Rf = 0.28 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.61-2.81 (m, 2H), 2.96-3.09 (m, 2H), 3.38 (dd, 1H, *J* = 14.4 Hz, *J* = 4.2 Hz), 6.01 (s, 2H), 6.72 (s, 1H), 7.03 (s, 1H), 7.17 (d, 1H, *J* = 5,1 Hz), 8.36 (d, 1H, *J* = 5.1 Hz), 8.61 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 32.0, 36.3, 46.8, 102.3, 102.4, 105.6, 123.4, 125.4, 130.5, 148.1, 148.2, 148.4, 150.6, 152.0, 154.6, 204.3. HRMS (ESI^+) *m/z* 346.0083 $[M+H]^+$, calcd for $C_{16}H_{13}BrNO_3$ 346.0079.

*2-[(3-Bromo-4-pyridyl)methyl]-5-chloro-indan-1-one (**9f**).* The title compound **9f** was prepared according to the general procedure E with compound **7f** (67 mg, 0.2 mmol) and Pt/C (5 wt% loading, 6.7 mg) as reactants. White solid. (Yield 67 mg, 99%). Rf = 0.54 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.79-2.87 (m, 2H), 3.07-3.23 (m, 2H), 3.45 (dd, 1H, *J* = 14.1 Hz, *J* = 4.5 Hz), 7.21 (d, 1H, *J* = 4.8 Hz), 7.35 (dd, 1H, *J* = 8.4 Hz, *J* = 1.8 Hz), 7.40 (d, 1H, *J* = 0.6 Hz), 7.69 (d, 1H, *J* = 8.1 Hz), 8.42 (d, 1H, *J* = 4.8 Hz), 8.68

(s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 31.9, 36.1, 46.7, 123.4, 125.3, 125.6, 126.8, 128.6, 134.6, 141.7, 147.9, 148.3, 152.1, 154.4, 205.0.

General procedure F for synthesis of compounds 10a-f. A toluene solution (3 mL) of compound **10a-f** (1 mmol), $Pd(dba)_2$ (0.04 mmol) and PPh_3 (0.08 mmol) was stirred at room temperature under argon for 15 min. Then, (1-ethoxyvinyl)tri(*n*-butyl)stannane (1-1.2 mmol) in toluene (3 mL) was added and the resulting mixture was stirred overnight at 110°C, cooled to room temperature and filtered on Celite®, washed with EtOAc and concentrated under reduced pressure. Purification on silica gel (gradient of EtOAc in Petroleum ether) afford a crude residue which was placed in presence of an aqueous solution of HCl 1M for 30 minutes at room temperature. After neutralization with a saturated aqueous solution of $NaHCO_3$, aqueous phase was extracted with dichloromethane (3x) and the combined organic layers were dried over magnesium sulfate to afford compound **10a-f** which were used without further purification.

*2-[(3-Acetyl-4-pyridyl)methyl]-5,6-dimethoxy-indan-1-one (**10a**).* The title compound **10a** was prepared according to the general procedure F with compound **9a** (120 mg, 0.33 mmol) and (1-ethoxyvinyl)tri(*n*-butyl)stannane (0.12 mL, 0.46 mmol) as reactants. White solid. (Yield 94 mg, 87%). Rf = 0.1 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.67 (s, 3H), 2.74-2.80 (m, 1H), 3.05-3.18 (m, 3H), 3.48-3.53 (m, 1H), 3.91 (s, 3H), 3.95 (s, 3H), 6.82 (s, 1H), 7.18 (s, 1H), 7.30 (d, 1H, *J* = 5.1 Hz), 8.60 (d, 1H, *J* = 5.1 Hz), 8.97 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 29.8, 32.0, 34.2, 47.7, 56.0, 56.1, 104.2, 107.2, 126.2, 128.8, 133.3, 148.5, 149.4, 149.6, 150.6, 151.9, 155.5, 199.9, 205.5. HRMS (ESI^+) *m/z* 326.1378 $[M+H]^+$, calcd for $C_{19}H_{20}NO_4$ 326.1392.

*2-[(3-Acetyl-4-pyridyl)methyl]-5-methyl-indan-1-one (**10b**).* The title compound **10b** was prepared according to the general procedure F with compound **9b** (750 mg, 2.38 mmol) and (1-

ethoxyvinyl)tri(*n*-butyl)stannane (0.8 mL, 2.38 mmol) as reactants. White solid. (Yield 398.8 mg, 60%). Rf = 0.11 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.43 (s, 3H), 2.67 (s, 3H), 2.83 (dd, 1H, *J* = 16.5 Hz, *J* = 3 Hz), 3.02-3.23 (m, 3H), 3.49-3.58 (m, 1H), 7.17-7.21 (m, 2H), 7.31-7.33 (m, 1H), 7.65 (d, 1H, *J* = 7.5 Hz), 8.61 (d, 1H, *J* = 5,1 Hz), 8.99 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 22.0, 29.8, 32.2, 34.1, 47.7, 123.8, 126.2, 126.8, 128.8, 133.3, 133.9, 146.1, 149.5, 150.6, 151.9, 153.6, 199.9, 206.4.

*2-[(3-Acetyl-4-pyridyl)methyl]-5-methoxy-indan-1-one (**10c**).* The title compound **10c** was prepared according to the general procedure F with compound **9c** (235 mg, 0.71 mmol) and (1-ethoxyvinyl)tri(*n*-butyl)stannane (0.34 mL, 0.99 mmol) as reactants. White solid. (Yield 135 mg, 64%). Rf = 0.17 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.61 (s, 3H), 2.76 (dd, 1H, *J* = 16.8 Hz, *J* = 3.0 Hz), 2.99-3.17 (m, 3H), 3.39-3.49 (m, 1H), 3.82 (s, 3H), 6.79 (s, 1H), 6.85 (dd, 1H, *J* = 8.4 Hz, *J* = 2.1 Hz), 7.27 (d, 1H, *J* = 4.8Hz), 7.63 (d, 1H, *J* = 8.4 Hz), 8.55 (d, 1H, *J* = 4.8 Hz), 8.92 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 29.9, 32.4, 34.2, 47.8, 55.6, 109.6, 115.5, 125.7, 126.3, 129.4, 133.5, 149.6, 150.6, 151.9, 156.2, 165.5, 200.0, 205.1. HRMS (ESI^+) *m/z* 296.1284 $[M+H]^+$, calcd for $C_{18}H_{18}NO_3$ 296.1287.

*2-[(3-Acetyl-4-pyridyl)methyl]-5,6-dimethyl-indan-1-one (**10d**).* The title compound **10d** was prepared according to the general procedure F with compound **9d** (235 mg, 0.71 mmol) and (1-ethoxyvinyl)tri(*n*-butyl)stannane (0.24 mL, 0.71 mmol) as reactants. White solid. (Yield 125.0 mg, 60%). Rf = 0.13 (EtOAc/Petroleum ether = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.27 (s, 3H), 2.29 (s, 3H), 2.63 (s, 3H), 2.74 (dd, 1H, *J* = 16.8 Hz, *J* = 3.0 Hz), 2.96-3.16 (m, 3H), 3.49 (dd, 1H, *J* = 12.3 Hz, *J* = 4.5 Hz), 7.14 (s, 1H), 7.27 (d, 1H, *J* = 5.1 Hz), 7.48 (s, 1H), 8.57 (d, 1H, *J* = 5.1 Hz), 8.94 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.8, 20.8, 29.9, 32.0, 34.2, 47.9,

124.3, 126.3, 127.2, 133.5, 134.4, 136.5, 145.3, 149.7, 150.7, 151.4, 200.0, 206.8. HRMS (ESI^+) *m/z* 294.1486 $[M+H]^+$, calcd for $C_{19}H_{20}NO_2$ 294.1494.

*6-[(3-Acetyl-4-pyridyl)methyl]-5,6-dihydrocyclopenta[f][1,3]benzodioxol-7-one (**10e**).* The title compound **10e** was prepared according to the general procedure F with compound **7e** (235 mg, 0.68 mmol) and (1-ethoxyvinyl)tri(*n*-butyl)stannane (0.32 mL, 0.95 mmol) as reactants. White solid. (Yield 135.0 mg, 64%). Rf = 0.12 (EtOAc/Petroleum ether = 3:2). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.61 (s, 3H), 2.63-2.71 (m, 1H), 2.98-3.11 (m, 3H), 3.40 (dd, 1H, *J* = 17.1 Hz, *J* = 6.3 Hz), 6.01 (s, 2H), 6.71 (s, 1H), 7.02 (s, 1H), 7.25 (d, 1H, *J* = 5,1 Hz), 8.55 (d, 1H, *J* = 5.1 Hz), 8.91 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 29.9, 32.4, 34.2, 48.1, 102.2, 102.5, 105.6, 126.3, 130.7, 133.4, 148.3, 149.6, 150.7, 150.8, 152.0, 154.4, 200.0, 204.8. HRMS (ESI^+) *m/z* 310.1073 $[M+H]^+$, calcd for $C_{18}H_{16}NO_4$ 310.1079.

*2-[(3-Acetyl-4-pyridyl)methyl]-5-chloro-indan-1-one (**10f**).* The title compound **10f** was prepared according to the general procedure F with compound **7f** (317 mg, 0.94 mmol) and (1-ethoxyvinyl)tri(*n*-butyl)stannane (0.31 mL, 0.94 mmol) as reactants. White solid. (Yield 221 mg, 78%). Rf = 0.15 (EtOAc/Petroleum ether = 3:2). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.66 (s, 3H), 2.85 (dd, 1H, *J* = 16.8 Hz, *J* = 3.3 Hz), 3.06-3.25 (m, 3H), 3.46-3.55 (m, 1H), 7.28-7.40 (m, 3H), 7.68 (d, 1H, *J* = 8.1 Hz), 8.61 (d, 1H, *J* = 5,1 Hz), 8.99 (s, 1H).

*(2,4,6-Trichlorophenyl) 4-[(5,6-dimethoxy-1-oxo-indan-2-yl)methyl]pyridine-3-carboxylate (**11**).* This compound was prepared using the method described by Manabe et al.[65] In a Schlenk tube under argon atmosphere, *n*-tributylamine (0.52 mL, 2.2 mmol) in anhydrous toluene (5 mL) was added to a mixture of Xantphos (38 mg, 0.06 mmol), compound **9a** (400 mg, 1.1 mmol) and $Pd(OAc)_2$ (7 mg, 0.03 mmol). The Schlenk tube was sealed with a Teflon cap and the reaction mixture was stirred at 100 °C for 5 minutes. Then, a degassed solution of 2,4,6-trichlorophenyl-

formate (498 mg, 2.2 mmol) in anhydrous toluene (10 mL) were added to the reaction mixture and heated overnight to 110°C. After cooling, the resulting suspension was filtered on Celite® and the filtrate was concentrated to dryness. The residue was purified by column chromatography on SiO_2 (gradient of EtOAc in petroleum ether) to afford the title compound **11** as a pale brown solid (Yield 312.0 mg, 55%). Rf = 0.36 (EtOAc/Petroleum ether = 7:3). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.74-2.82 (m, 1H), 3.05-3.29 (m, 3H), 3.62-3.68 (m, 1H), 3.89 (s, 3H), 3.93 (s, 3H) 6.78 (s, 1H), 7.15 (s, 1H), 7.39 (d, 1H, *J* = 5.1 Hz), 7.42 (s, 2H), 8.72 (d, 1H, *J* = 5.1 Hz), 9.42 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 32.1, 34.4, 48.0, 56.2, 56.3, 104.5, 107.4, 124.0, 126.2, 128.8, 128.9, 129.7, 132.6, 142.7, 148.5, 149.7, 152.5, 152.6, 153.5, 155.8, 162.4, 205.2. HRMS (ESI^+) *m/z* 506.0339 $[M+H]^+$, calcd for $C_{24}H_{19}Cl_3NO_5$ 506.0329.

*Methyl 4-[(5,6-dimethoxy-1-oxo-indan-2-yl)methyl]pyridine-3-carboxylate (**12**).* To a solution of ester **11** (130 mg, 0.236 mmol) in anhydrous MeOH (5 mL) was added NEt_3 (66µL, 0.472 mmol) at room temperature. Then, the mixture was stirred at reflux and after 17 h the reaction mixture was evaporated to dryness, taken up in diethyl ether. The resulting solid was filtered, rinsed twice with diethyl ether and dried under vacuum to afford the compound **12** (60 mg, 75%) as a pale yellow solid. Rf = 0.18 (EtOAc/Petroleum ether = 7:3). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.74-2.79 (m, 1H), 3.06-3.15 (m, 3H), 3.67-3.71 (m, 1H), 3.91 (s, 3H), 3.94 (s, 6H) 6.82 (s, 1H), 7.19 (s, 1H), 7.27-7.29 (m, 1H), 8.62 (d, 1H, *J* = 5.1 Hz), 9.10 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 32.0, 34.6, 48.0, 52.4, 56.1, 56.3, 104.4, 107.4, 125.8, 126.0, 129.0, 148.5, 149.6, 151.0, 152.0, 152.3, 155.7, 166.5, 205.4. HRMS (ESI^+) *m/z* 342.1343 $[M+H]^+$, calcd for $C_{19}H_{20}NO_5$ 342.1341.

*4-[(5,6-Dimethoxy-1-oxo-indan-2-yl)methyl]-N-methyl-pyridine-3-carboxamide (**13**).* In a Schlenk tube under argon atmosphere, to a solution of ester **11** (100 mg, 0.182 mmol) in

anhydrous THF (2 mL) was added NEt_3 (50µL, 0.36 mmol) and methylamine solution 2M in THF (182 µL, 0.36 mmol) at room temperature. The Schlenk tube was sealed with a Teflon cap and the reaction mixture was stirred at 70 °C for 1h. After cooling at room temperature, the reaction mixture was evaporated to dryness and taken up in diethyl ether. The resulting solid was filtered, rinsed with diethyl ether and dried under vacuum to afford the compound **13** (60 mg, 99%) as a pale yellow solid. Rf = 0.25 (CH_2Cl_2/MeOH = 96:4). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.77-3.11 (m, 4H), 2.90 (d, 3H, *J* = 4.8 Hz), 3.25 (dd, 1H, *J* = 12.9 Hz, *J* = 4.2 Hz), 3.83 (s, 3H), 3.87 (s, 3H), 6.76 (s, 1H), 6.92 (d, 1H, *J* = 4.5 Hz), 7.06 (s, 1H), 7.15 (d, 1H, *J* = 4.8 Hz), 8.38 (d, 1H, *J* = 4.5 Hz), 8.46 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 26.7, 32.1, 33.3, 47.9, 56.0, 56.2, 104.2, 107.4, 124.7, 128.7, 133.1, 147.8, 148.9, 149.5, 150.4, 155.8, 168.1, 205.8.

*4-[(5,6-Dimethoxy-1-oxo-indan-2-yl)methyl]pyridine-3-carboxamide (**14**).* In a Schlenk tube under argon atmosphere, a solution of 0.5M ammonia in dioxane (6 mL) was added to ester **11** (400 mg, 0.789 mmol) at room temperature. The Schlenk tube was sealed with a Teflon cap and the reaction mixture was stirred at 80 °C for 17h. After cooling at room temperature, the reaction mixture was evaporated to dryness and taken up in diethyl ether. The resulting solid was filtered, rinsed with diethyl ether and dried under vacuum to afford the compound **14** (260 mg, 78%) as a pale yellow solid. Rf = 0.05 (EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.84-2.89 (m, 1H), 3.02-3.20 (m, 3H), 3.22-3-42 (m, 1H), 3.90 (s, 3H), 3.95 (s, 3H), 5.80 (br s, 1H), 6.19 (br s, 1H), 6.83 (s, 1H), 7.15 (s, 1H), 7.28 (d, 1H, *J* = 5.7 Hz), 8.57 (d, 1H, *J* = 5.7 Hz), 8.71 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 31.4, 32.9, 47.1, 55.6, 56.0, 104.0, 108.2, 124.8, 128.1, 133.1, 146.9, 147.7, 148.7, 149.2, 150.1, 155.4, 168.9, 204.9. HRMS (ESI^+) *m/*z 327.1337 $[M+H]^+$, calcd for $C_{18}H_{19}N_2O_4$ 327.1345.

*4-[(5,6-Dimethoxy-1-oxo-indan-2-yl)methyl]pyridine-3-carbonitrile (**15**).* This compound was prepared using the method described by Enthaler and Weidauer.[66] In a Schlenk tube under argon atmosphere and at room temperature, compound **14** (82 mg, 0.25 mmol) and $CuCl_2$ (1.6 mg, 0.012 mmol) were placed in suspension in anhydrous toluene (2 mL) then MSTFA (139 μL, 0.75 mmol) was added. The Schlenk tube was sealed with a Teflon cap and the reaction mixture was stirred at 100 °C for 5 hours. After cooling to room temperature, a saturated solution of ammonium chloride was added and the mixture was extracted with EtOAc (3x). The combined organic phases were washed with a saturated solution of ammonium chloride (1x) and brine (1x), dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by column chromatography on SiO_2 (gradient of EtOAc in petroleum ether) to afford the title compound **15** as a pale yellow solid (Yield 65.0 mg, 83%). Rf = 0.41 (EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.78 (dd, 1H, *J* = 16.8 Hz, *J* = 3.0 Hz), 3.07-3.10 (m, 2H), 3.21 (dd, 1H, *J* = 16.5 Hz, *J* = 6.6 Hz), 3.43-3-52 (m, 1H), 3.92 (s, 3H), 3.96 (s, 3H), 6.84 (s, 1H), 7.19 (s, 1H), 7.39 (d, 1H, *J* = 5.1 Hz), 8.70 (d, 1H, *J* = 5.1 Hz), 8.85 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 31.7, 34.8, 47.5, 56.1, 56.3, 104.3, 107.3, 110.9, 116.0, 124.4, 128.5, 148.2, 149.7, 152.6, 152.7, 153.0, 155.9, 204.2. HRMS (ESI^+) *m/z* 309.1230 $[M+H]^+$, calcd for $C_{18}H_{17}N_2O_3$ 309.1239.

In Vitro Biological Studies. **AChE and BChE Inhibition Assay.** Inhibitory capacity of compounds on AChE biological activity was evaluated through the use of the spectrometric method of Ellman.[53] Acetyl- or butyrylthiocholine iodide and 5,5-dithiobis-(2-nitrobenzoic) acid (DTNB) were purchased from Sigma Aldrich. Lyophilized electric eel AChE (Type III, Sigma Aldrich), or BChE from equine serum (Sigma Aldrich) was dissolved in 0.2 M phosphate buffer pH 7.4 such as to have enzyme solutions stock with 2.5 units/mL enzyme activity. AChE from human erythrocytes (buffered aqueous solution, ≥500 units/mg protein (BCA), Sigma Aldrich)

was diluted in 20 mM HEPES buffer pH 8, 0.1% Triton X-100 such as to have enzyme solution with 0.25 units/mL enzyme activity. In the procedure, 100 µL of 0.3 mM DTNB dissolved in phosphate buffer pH 7.4 were added into the 96 wells plate followed by 50 µL of the tested compound solution and 50 µL of enzyme solution. After 5 min of preincubation, the reaction was then initiated by the injection of 50 µL of 10 mM acetyl- or butyrylthiocholine iodide solution. The hydrolysis of acetyl- or butyrylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetyl- or butyrylthiocholine, at a wavelength of 412 nm every minute for 10 min using a 96-well microplate plate reader (TECAN Infinite M200, Lyon, France). Tested compounds were dissolved in analytical grade DMSO. Donepezil was used as reference standard. First screening of electric eel AChE, BChE activity and AChE from human erythrocytes activity were carried out at a 10^{-5} M, 10^{-5} M and at 10^{-6} M concentration of compounds respectively under study. For the compounds with significant inhibition (≥ 50%) after 4 min of reaction, IC_{50} values were determined graphically from 6 points inhibition curves using the Origin® software.

Propidium Competition Assay. Propidium exhibits an increase in fluorescence on binding to AChE peripheral site, making it a useful probe for competitive ligand binding to the enzyme. Fluorescence was measured in a Tecan Infinite® M200 plate reader. Measurements were carried out in 200 µl solution volume, in 96-well plates. The buffer used was 1 mM Tris/HCl, pH 8.0, 5 U EeAChE which was incubated, for 6 hours at 25°C, with a 150 µL 10^{-5}M solution of the compounds or donepezil (from Tocris) as control. One micromolar propidium iodide 50 µL solution was added 10 min before fluorescence measurement. The excitation wavelength was 535 nm, and that of emission, 595 nm. Each assay was repeated, at least, at three different times.

***h*AChE Kinetic Study with Compound 2r.** The kinetic studies were performed with compound **2r** using the spectrometric Ellman's method[53] at different concentrations of the substrate acetylthiocholine iodide (0.04 – 0.2 mM for each concentration of tested compound **2r**). Four concentrations of **2r** were used for the studies: 1, 10, 100 and 1000 nM. Each experiment was performed in triplicate. V_{max} and K_m values of the Michaelis-Menten kinetics were calculated by nonlinear regression from substrate-velocity curves. Lineweaver-Burk plots were calculated using linear regression in GraphPad Prism® 5.

Inhibition of AChE-induced Aβ (1–40) aggregation in the presence of 2r.[67] Recombinant human HFIP-pretreated Aβ1–40 peptide (Bachem AG, Switzerland) was dissolved in DMSO to give 230 μM stock solution. To 2 μL of previous stock solution was added human recombinant AChE in 0.215 M sodium phosphate buffer pH=8.0 (final concentration of 2.30 μM, ratio 100:1) or hrAChE in the presence of propidium iodide or compound **2r** in 0.215 M sodium phosphate buffer pH=8.0 (final concentration of 100 μM). Blanks with Aβ1–40 peptide or hrAChE, both with or without tested compound in 0.215 M sodium phosphate buffer pH=8.0 were also prepared. The final volume for each experiment was 20 μL. Assay was run in duplicate. After 24h of incubation at room temperature, the amyloid fibril formation was quantify by the thioflavine T (ThT) fluorescence method.[68] Samples were diluted to 2 mL with a 50 mM glycine NaOH buffer pH=8.5 containing 1.5 μM of ThT and the fluorescence was measured (excitation wavelength 446nm, emission wavelength 485 nm). Background fluorescence of 1.5 μM ThT was subtracted. The hrAChE induced Aβ1–40 aggregation inhibitory potency is expressed as the percentage inhibition (% inh= 100 – (IFi / IF0 ×100%), where IFi and F0 are respectively the fluorescence intensities obtained for Aβ1–40 plus hrAChE int the presence or in the absence of inhibitor.

Inhibition of Self-induced Aβ(1–42) Aggregation in the presence of 2r. The SensoLyte® ThT β-Amyloid (1-42) Aggregation kit provides a convenient and standard method to measure Aβ42 aggregation using Thioflavin T dye. Aβ42 peptide is pretreated to ensure it is in a monomeric state. An optimized fibrillation buffer is included with the kit, and two known inhibitors are supplied as controls. The protocol used is the one indicated by the supplier (β amyloid aggregation Sensolyte® Thioflavin kit; Anaspec ref: 72214). Briefly, stock solution of the studied compounds **2r** is prepared at a concentration of 1 mM in DMSO. Then, 1 µL of the solution is placed in a 96 well plate (Greiner ref 655900). So, 10 µL of the thioflavineT solution (2 mM) and 85 µL of the solution of the amyloid β-peptide 1-42 (250 µg/mL in the supplied buffer) are added to each well. The reaction volume was made up to 100 µL with the reaction buffer supplied by the kit. In parallel, a positive control is performed without compound and references are tested (curcumin, 10 µM / morin and phenol red, 100 µM). Each compound is tested in triplicate. The fluorescence is measured every 5 min at 37°C (λ_{ex}/ λ_{em} = 440 nm/484 nm) with the InfiniteR M200 reader (Tecan). The calculation of percent inhibition is performed as follows: 100 - (I_{Fi} / I_{FO} x 100) with I_{Fi} corresponding to the fluorescence measured in the presence of the tested compound and I_{FO} without the tested compound.

Blood-Brain Barrier Permeability. The PAMPA-BBB test is performed according to the methodology using the BBB-Pampa Explorer® kit (*p*ION Inc, Woburn, MA, USA). Compounds were diluted to 20 mM in DMSO and then at 100 µM in pH 7.4 Prisma HT buffer (*p*ION). Then, 200 µL of this solution were added to each well of the donor plate (n = 6). The filter membrane on the acceptor plate was coated with 5 µL of the BBB-1 lipid (*p*ION) formulation and to each well of the acceptor plate, 200 µL of brain sink buffer (BSB, *p*ION) was added. The acceptor filter plate was placed on top of the donor plate to form a "sandwich". The sandwich was

incubated at room temperature for 4h without stirring. The sandwich is then separated and the UV-vis spectra were measured in the reference, acceptor and donor plates using a microplate reader (Tecan infinite M200). For each compound, -logP_e was calculated using the PAMPA Explorer® software v. 3.7 (*p*ION). The references used are theophylline (250 μM) and corticosterone (100 μM).

Studies of Stability/Oxidation of 1r. The in vitro stability of the test compound **1r** was studied in 10% methanolic solution of $AgNO_3$, in PBS buffer (pH 7.4), in 100% of rehydrated human plasma with PBS buffer (pH 7.4), in 2% NAD^+ solution in PBS, in 2% (-)-Riboflavin solution in PBS and in freshly prepared 10% mice brain homogenate in PBS (pH 7.4). The incubation was initiated by the addition of the test compound **1r** (10 mM in DMSO) to preheated $AgNO_3$, PBS, plasma, 2% NAD^+, 2% riboflavin and brain homogenate solutions to obtain a final concentration of 25 μM. The assays were performed at 37°C and conducted in duplicate. A standard curve for **1r** and **2r** of six points was made to fit the measured concentrations. Samples (100 μL) were taken at appropriate time intervals (6 points) and added to 100 μL acetonitrile containing internal standard. Samples (10 μL) in PBS, 2% NAD^+ or riboflavin solutions were directly analyzed by HPLC-UV, samples (10 μL) in methanolic solution of $AgNO_3$ were analyzed after centrifugation and samples in human plasma and in mice brain homogenate were subjected to vortex mixing for 1 min and then centrifugation for 10 min at 13,000 rpm in order to deproteinize. Samples (10 μL) of the resulting supernatants were withdrawn and analyzed by HPLC-UV for determine the percentage of remaining prodrug **1r** and oxidized compound **2r**. The values represent the mean of two independent experiments. The in vitro plasma half-life ($t_{1/2}$) was calculated using the expression $t_{1/2}$=0.693/b, where b is the slope found in the linear fit of the natural logarithm of the fraction remaining of the parent compound *vs* incubation time.

In Vitro DPPH Radical Scavenging Assays. DPPH radical scavenging activity was determined according to the method described by Blois.[69] 0.5 mL of 0.1 mM DPPH radical solution in methanol was mixed with 1.5 mL of various concentrations in methanol of **1r**, Donepezil or ascorbic acid (used as positive control). The mixture was then thoroughly vortexed for 5s and each antioxidant-DPPH radical reaction mixture is kept in the test tubes in dark at 37°C for 40 min (time point by which all reactions should have reached steady state) and transferred to a cuvette for absorbance measurement at 517 nm. For the baseline control, 1.5 mL of methanol was used. Mean values were obtained from triplicate experiments. Percentage of inhibition was calculated using the equation ([A0−A1]/A0) × 100 where A0 was the absorbance of the control and A1 was the absorbance in the presence of the compound. Percent inhibition was plotted *vs* concentration; the EC 50 values were determined using XLfit®.

In Vitro Genotoxicity Tests. Ames fluctuation assays were performed by Toxem (France).[70-72] *His*-Bacteria cells (*ca* 10^7) of TA98 and TAMix strains from *Salmonella typhimurium* were tested for 90 minutes at six concentrations with concurrent zero-dose and positive controls. Assays were conducted with or without a metabolic mammalian activation system. Cells culture was run by including a pH indicator lacking histidine. The cells culture was aliquoted into 48-wells of a 384-well plate. Within 48h, cells have undergone the reversion to histidine prototrophy (mutant histidine revertant *His*+) will grow into colonies. Bacterial metabolism reduces the pH of the medium, changing the color of that well. The frequency of mutation is counted as the number of wells out of 48 which have changed. Each dose is done in triplicate to allow for statistical analysis of the data. An increase in the number of revertant colonies upon exposure to test chemical relative to controls indicates that the chemical is mutagenic.

Cell Viability Assay on Human Neuroblastoma SH-SY5Y. Briefly, human neuroblastoma SH-SY5Y cells were seeded into 24-well plates at a density of $3x10^4$ cells/well and incubated for 24h to ensure cell adhesion. Cells were then treated and incubated with 0.1 to 1 mM (final concentration) of compound **2r**, donepezil or MPP+ in PBS buffer for 24h at 37°C and cell viability was assessed with 1.3 µM calcein-AM (molecular Probes) in PBS for 15 min. After washing twice with PBS, fluorescence measurements were performed with an Infinite 200 microplate reader (Tecan) at an excitation/emission wavelengths at 485/530 nm. The control group (without compound) was considered as 100% cell viability. Data are means ±SD of at least two independent experiments, each carried out in four replicates.

In Vivo Biological Study. **Evaluation of Acute Toxicity of 1r and 2r.** The solutions were formulated so that animals were injected with a maximal dosing volume of 5 mL/kg. The appropriate amount according to the dosage was mixed into a 4% DMSO – 8 % Tween 80 in normal saline solution (v/v/v). The mixtures were then vortexed and stored in a sonication bath until time of injection. Then, Lorke's method was used to determine LD50.[73] All of the animal procedures followed the guidelines for the care and handling of laboratory animals and were approved by the Ethics Committee of the Faculty of Pharmacy in Rouen. CD1 Female mice weighing 20-25 g were obtained from Charles River Laboratories (France). The animals were kept in a temperature-controlled environment (air-conditioning, T= 21 ± 1° C with a 12 h dark/light cycle and food and water were freely available. For each compounds, the animals were randomly divided into 6 groups of 3 animals. Each group of animals are administrated different doses in formulation of test compounds. The animals were observed during 72 h after administering the test compound with following of any toxicity associated with cardiac, neurological and behavioral such as tremors, convulsions, salivation, diarrhea, sleep, lacrimation,

and feeding behavior in treated mice as a sign of acute toxicity. The geographic mean of the least dose that killed mice (LD100) and the highest dose that did not induced mortality (LD0) was taken as the LD50.

Repeated Dose 14-Day i.p. Toxicity of 1r in CD1 Male Mice. Compound **1r** (10 mg/kg) was administered by ip to CD1 male mice (38 ± 8 g) from Charles River Laboratories (France). All of the animal procedures followed the guidelines for the care and handling of laboratory animals and were approved by the Ethics Committee of the Faculty of Pharmacy in Rouen. The animals were kept in a temperature-controlled environment (air-conditioning, T= 21 ± 1°C) with a 12 h dark/light cycle and food and water were freely available. Fifteen mice were randomly divided into three groups. To the first group, compound **1r** in formulation (4% DMSO, 8% Tween 80, 88% normal saline solution) was administered daily by i.p. (n= 5, 10 mg/kg) for 14 days. To the second group, compound **1r** in formulation (4% DMSO, 8% Tween 80, 88% normal saline solution) was administered once by i.p. (n= 5, 10 mg/kg). Then an equivalent volume of formulation was administered by i.p. to the control group (n= 5). Any changes in locomotor activity were recorded for a total of 180 min after each administration of test compound or formulation. They *were weighed daily* and observed for mortality for 14 days for group 1 and control. At the end of this behavioral study (14 days for groups 1 and control, 24h for group 2), each animal was euthanized and the blood and brain tissues were collected for the evaluation of exposure levels of **1r** and **2r** by LC-MSMS.

ASSOCIATED CONTENT

The Supporting Information is available free of charge via the Internet at http://pubs.acs.org. Experimental procedures and compound characterization data for compounds **1**,**2** and in vitro/in

vivo pharmacology procedures. Docking procedures. PDB files of compounds **1r** (*R,R*) and (*S,S*) enantiomers with 4EY7. PDB files of compounds **2r** (*R*) and (*S*) enantiomers with 4EY7.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

Aβ, β-amyloid peptide; AChE, acetylcholinesterase; AChEI, acetylcholinesterase inhibitor; ACN, acetonitrile; AD, Alzheimer's disease; ATC, acetylthiocholine; BBB, blood-brain barrier; BChE, butyrylcholinesterase; BNAH, *N*-benzyl-1,4-dihydro-nicotinamide; CAS, catalytic anionic site; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; *Ee*AChE, electric eel acetylcholinesterase; *eq*BChE, equine butyrylcholinesterase; ESI^+, positive electrospray ionization; EWG, electron withdrawing group; *h*AChE, human acetylcholinesterase; *h*AChEI, human acetylcholinesterase inhibitor; HRMS, high-resolution mass spectrometry; IC_{50}, half maximal inhibitory concentration; LD_{50}, median lethal dose; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; NAD^+, nicotinamide adenine dinucleotide (oxidized form); NADH, nicotinamide adenine dinucleotide (reduced form); NMR, nuclear magnetic resonance;

PAMPA, parallel artificial membrane permeability assay; PAS, peripheral anionic site; PBS, phosphate-buffered saline; PDB, protein data bank; PTSA, *p*-toluenesulfonic acid; Rf, retention factor; r*h*AChE, recombinant human acetylcholinesterase; SAR, structure-activity relationship; SD, standard deviation; THF, tetrahydrofuran; ThT, thioflavin T; TLC, thin-layer chromatography.

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