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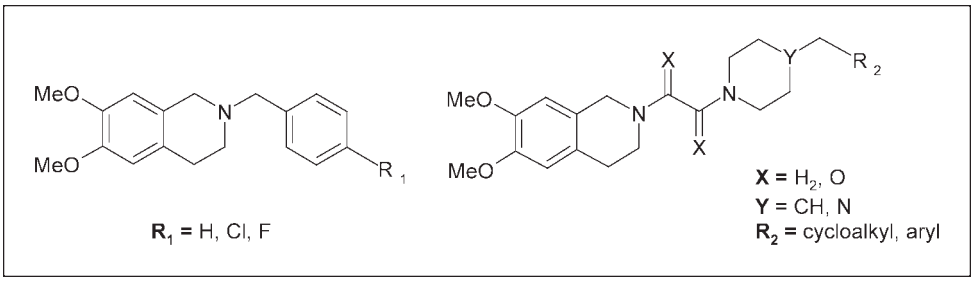

N-substituted donepezil-related isoquinolines have been prepared as potential acetylcholinesterase inhibitors (AChEIs). Microwave assisted procedures and solution-phase parallel synthesis were chosen to optimize the synthetic approach and improve the yields. All synthesized compounds were tested for their AChE inhibitory activity by colorimetric Ellman method and some of them (**10**, **13**, and **28**) displayed low inhibitory effects at μ*M* concentrations.

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INTRODUCTION

Alzheimer's disease (AD), the most common cause of senile dementia, is a neurodegenerative disorder characterized by loss of cognitive ability and severe behavioral abnormalities, which become increasingly serious with disease progression [1].

AD is mainly characterized by a pronounced degeneration of the cholinergic system and by the alteration of other neurotransmitter systems such as the glutamatergic system. Cholinergic abnormalities are associated with (a) the accumulation of protein deposits as β-amyloid peptide (Aβ), the main component of the senile plaques and derived from amyloid precursor protein (APP); (b) the abnormal phosphorylation of τ protein resulting in the formation of neurofibrillary tangles (NFT).

As the cause of the disease has yet to be identified, despite the numerous drugs have been studied as potential anti-AD agents, its treatment has been confined to limiting the progression of the disease. Until now, drug development has focused on (i) symptomatic treatments for restoring deficient neurotransmitters and (ii) etiologically based treatments for slowing or halting the rate of progression [2,3].

Current strategies in the search for new therapeutic approaches are based on different morphological and biochemical characteristics of AD and focus on the following directions: (i) agents compensating the cholinergic system hypofunction; (ii) agents interfering with the metabolism of Aβ; (iii) agents affecting the process of τ protein iperphosphorylation and formation of NFT; (iv) agents protecting neurons from toxic metabolites formed in neurodegenerative processes; and (v) agents activating other neurotransmitter systems to indirectly compensate the cholinergic function deficit.

Currently, only a few therapeutic drugs are available for the treatment of AD; their pharmacological effect is the enhancement of the central cholinergic function, by increasing brain acetylcholine (ACh) level [2,4–6].

Cholinergic therapy for Alzheimer's disease is mainly concentrated on the inhibition of acetylcholinesterase (AChE, EC 3.1.1.7), the main enzyme involved in the breakdown of acetylcholine in the normal brain [7,8]. Tacrine (**1**, Cognex®), donepezil (**2**, Aricept®), rivastigmine (**3**, Exelon®), and galantamine (**4**, Reminyl®) (Fig. 1) are the most widely used acetylcholinesterase inhibitors (AChEIs) [9]. The main effect of AChEIs on neurotransmission is thought to be associated with the increase of both the duration of action and the concentration of the neurotransmitter in the synaptic cleft, resulting in an improvement of the activation of the cholinergic receptors [10].

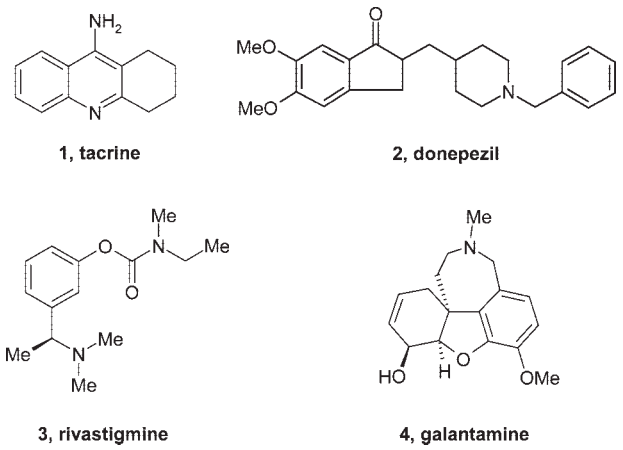


Figure 1. AChE inhibitors used for the treatment of AD.

Recently, it has been pointed out that AChE exerts secondary noncholinergic functions and it is also responsible for the pro-aggregating activity toward Aβ, thus suggesting that AChE plays a key role in the development of the senile plaques. In particular, the peripheral anionic binding site (PAS) of AChE is responsible for the AChE–Aβ interaction promoting amyloid fibril formation [11,12]. For these reasons AChEIs able to interact with both catalytic and peripheral binding sites (i.e., dual binding site inhibitors) represent a new therapeutic approach to counteract hypofunction of the cholinergic system and to avoid Aβ aggregation [13]. On the basis of this evidence several classes of dual binding site AChE inhibitors have been developed. Recently, different classes of such compounds have been designed from donepezil (**2**) [14–18]. As demonstrated by the crystal structure of their complex with *Torpedo californica* AChE (*Tc*AChE), **2** is able to occupy the entire length of the enzyme active-site gorge forming various interactions with specific residues, such as aromatic residues, stacking interactions between the benzyl and indanone moieties and the indole ring of Trp84 and Trp279 at the catalytic and peripheral sites, respectively, and the cation–π interaction between the piperidine nitrogen and the phenyl ring of Phe330 residue.

However, now it also seems that the role of butyrylcholinesterase (BChE, EC 3.1.1.8) in hydrolysing acetylcholine may be relevant in brain degenerative conditions. In fact, as Alzheimer disease progresses, AChE activity decreases in some brain regions, while BChE activity increases. This is probably related to a relative increase in the numbers of BChE-positive neurons, likely as compensation for AChE decrease. For these reasons, one of the most innovative approaches is the development of cholinesterase inhibitors (ChEIs), able to inhibit AChE and BChE [6].

We herein report the design, the synthesis and the cholinesterase (AChE and BChE) inhibitory activity of a new series of *N*-substituted 6,7-dimethoxyisoquinoline derivatives (**5–30**, Fig. 2) containing in their structures some moieties considered able to inhibit enzyme activity. The 6,7-dimethoxyisoquinoline nucleus and the benzyl substituent were chosen considering the donepezil chemical structure; furthermore, the linker was modified with the aim of evaluating the effect on the inhibitory potency of the distance between (i) the two aromatic rings that could be involved in stacking interactions with the indole ring of Trp84 and Trp279; (ii) the aromatic ring and the positive ionisable nitrogen atom of benzyl-piperidine or -piperazine moiety. Moreover, we inserted into the linker the carbonyl group that characterizes a useful interaction point for the donepezil-like derivatives.

All synthesized compounds were tested for their ability to inhibit AChE and some compounds were also evaluated against BChE.

RESULTS AND DISCUSSION

As shown in Scheme 1, the synthesis of designed compounds was performed starting from the commercially available 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**31**) that was functionalized to give the different *N*-substituted derivatives **5–29**.

Compounds **5–7** were easily prepared by reaction of derivative **31** with a suitable arylbromide under basic conditions. The reaction of isoquinoline **31** and ethyl oxalylchloride in the presence of triethylamine gave ethyl ester intermediate which was converted into derivative **32** by hydrolysis under basic conditions and then treatment with 6*N* HCl. Thus, 1-(4-benzylpiperidin-1-yl)-2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl) ethane-1,2-dione (**8**) was easily achieved by HBTU-mediated coupling of intermediate **32** with benzylpiperidine.

Also compounds **9–29** were prepared in two steps. In the first step, **31** was combined with 2-chloroacetyl chloride to give the key intermediate **33**, which in the second step was reacted with benzylpiperidine or 4-arylmethylpiperazine derivatives to provide compounds **9–29**. These reactions were carried out following a solution phase parallel

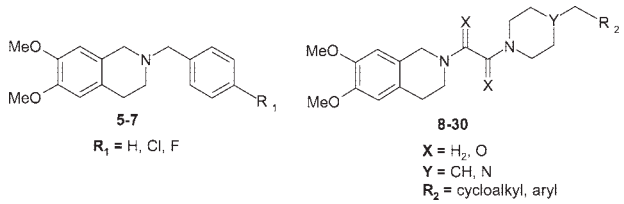
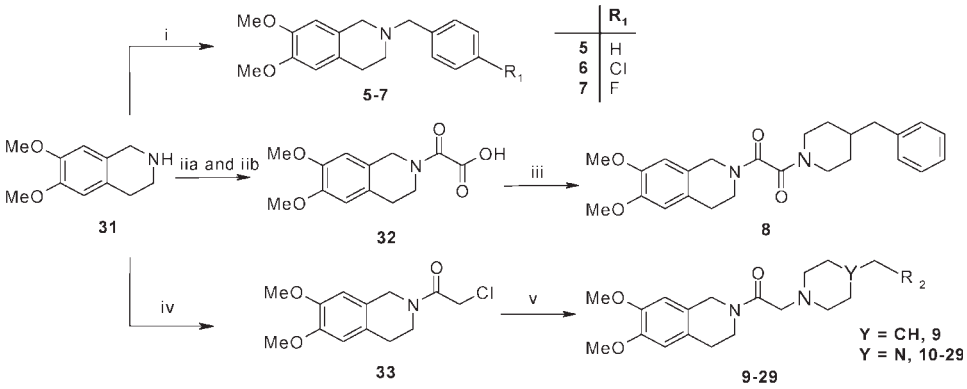


Figure 2. Designed molecules.

Scheme 1



Cpd	R_2
9	C_6H_5
10	C_6H_5
11	4-BrC_6H_4
12	4-ClC_6H_4
13	2-FC_6H_4
14	3-FC_6H_4
15	4-FC_6H_4
16	3-MeC_6H_4
17	4-MeC_6H_4
18	4-$OMeC_6H_4$
19	3-$CF_3C_6H_4$

Cpd	R_2
20	4-$CF_3C_6H_4$
21	4-$(Me_3C)C_6H_4$
22	3,4-$Cl_2C_6H_3$
23	2-Cl,6-FC_6H_3
24	2,4-$F_2C_6H_3$
25	2,4,6-$Me_3C_6H_2$
26	$CH_2C_6H_5$
27	CH–CH-C_6H_5
28	cyclohexyl
29	

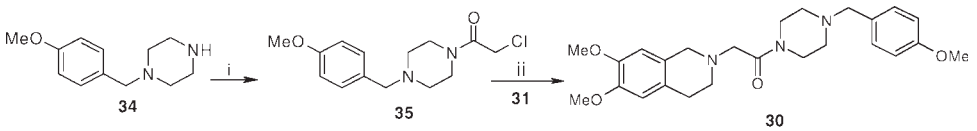
Reagents and conditions: i) $R_1C_6H_4CH_2Br$, TEA, DCM r.t., 24 h or NaH, DMF, r.t., 3 h; iia) ClCOCOOEt, TEA, DCM, 25°C, 5 min., 280 Watt; iib) KOH, EtOH/H_2O, HCl 6N; iii) HBTU, TEA, benzylpiperidine, DMF, 40 min, 25°C, 250 Watt; iv) $ClCOCH_2Cl$, TEA, DCM, 0°C, 1h; v) cycloalkylamine, TEA, DCM, Δ, 24h.

synthetic (SPPS) approach, using a Buchi Syncore reactor, which allowed us to prepare a small library of analogs.

As shown in Scheme 2, the synthesis of 2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)-1-[4-(4-methoxybenzyl)piperazin-1-yl]ethanone (**30**) was realized by using an alternative procedure. Compound **35** was prepared by the same method employed for compound **33** but starting from the 4-(4-methoxyphenyl)methyl-piperazine **34**. Then, the reactive intermediate **35** was combined with **31** to give **30** under basic conditions.

To evaluate the inhibitory activity, all compounds were prepared as hydrochloride derivatives (except compound **8**) and tested on human recombinant AChE according to Ellman [19], using tacrine (**1**) as reference compound. Unfortunately, only derivatives **10**, **13**, and **28** showed inhibitory effects at μ*M* concentrations. The IC_{50} values were 176 μ*M* (**10**), 102 μ*M* (**13**), and 148 μ*M* (**28**), respectively. These results showed that our compounds exhibited lower potency than **1** (IC_{50} = 424 n*M*) and **2** (IC_{50} = 23.1 n*M*) [20].

Scheme 2



Reagents and conditions: i) $ClCOCH_2Cl$, TEA, DCM, rt, 1h; ii) NaH, dry DMF, N_2, r.t., 20h.

Moreover, to explore the dual inhibitor effects of the most active compounds (**10**, **13**, and **28**), we performed the assay against the BChE enzyme at different concentrations (25–800 μ*M*). The results obtained suggest that these compounds did not significantly inhibit BChE; only compound **13** showed 82% of inhibitory activity at the highest tested dose (800 μ*M*).

In conclusion, microwave-assisted procedures and solution-phase parallel synthesis were set up to prepare new isoquinoline derivatives (**5–30**) as potential donepezil-like AChE inhibitors. These approaches allowed us to successfully obtain a small library of compounds that were tested as cholinesterase inhibitors. Some of the synthesized compounds showed inhibitory activity at μ*M* concentrations. However, these results are not useful to make structure-activity relationships for this new series of isoquinolines.

EXPERIMENTAL

Chemistry. All microwave-assisted reactions were carried out in a CEM Focused Microwave Synthesis System, Model Discover working at the potency necessary for refluxing under atmospheric conditions (i.e., 250–300 W). Melting points were determined on a BUCHI Melting Point B-545 apparatus and are uncorrected. Elemental analyses were carried out by University of Messina (C, H, N) using a Carlo Erba Model 1106 Elemental Analyzer and by Redox S.n.c. (Monza, Italy) (C, H, N, Cl). The obtained results are within ± 0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (230–400 mesh) and Flash Chromatography (FC) on a Biotage SP4 EXP. ^{1}H NMR spectra were recorded in deuterochloroform ($CDCl_3$) with TMS as internal standard or hexa-deutero-dimethylsulfoxide (DMSO-d_6) on a Varian Gemini-300 spectrometer. Chemical shifts were expressed in δ (ppm) and coupling constants (*J*) in Hz.

General procedure for the synthesis of 2-benzyl-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline derivatives (5–7). 1, 2,3,4-Tetrahydro-6,7-dimethoxyisoquinoline hydrochloride (229.70 mg, 1 mmol) was dissolved in water (10 mL) and alkalinized with a saturated aqueous solution of NaOH until pH = 10–11. The resulting mixture was extracted with ethyl acetate, dried over sodium sulfate (Na_2SO_4) and concentrated in vacuo to give the corresponding free-base **31**. Then 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (**31**) (193.25 mg, 1 mmol) was dissolved in dichloromethane (DCM) (10 mL) and triethylamine (TEA) (102.20 mg, 1 mmol) was added. The mixture was treated with the suitable benzyl bromide (1 mmol) and stirred for 24 h at room temperature. The reaction was quenched with water (10 mL), extracted with ethyl acetate (3 × 10 mL) and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was powdered by treatment with diethyl ether and crystallized from ethanol to give derivatives **5–7**. Compounds **5–7** were also obtained in higher yields using a catalytic amount of sodium hydride (NaH) in dimethylformamide (DMF) (2 mL). In this case, the reaction mixture was stirred at room temperature for 3 h and then a saturated aqueous $NaHCO_3$ solution was added. After the extraction with ethyl acetate (3 × 10 mL) the organic phase was reduced under vacuo, the residue powdered by treatment with diethyl ether and crystallized from ethanol to give the desired derivatives **5–7**.

2-Benzyl-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (5). Mp 85–87°C, yield 77%; ^{1}H NMR (δ in $CDCl_3$) : 2.72–2.83 (m, 4H, CH_2), 3.55 (s, 2H, CH_2), 3.68 (s, 2H, CH_2), 3.81 (s, 3H, CH_3O), 3.84 (s, 3H, CH_3O), 6.48 (s, 1H, ArH), 6.60 (s, 1H, ArH), 7.28–7.41 (m, 5H, ArH). Anal. Calcd for $C_{18}H_{21}NO_2$: C, 76.42; H, 7.50; N, 4.79. Found: C, 76.30; H, 7.47; N, 4.94.

2-(4-Chlorobenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (6). Mp 95–97°C, yield 52%; ^{1}H NMR (δ in $CDCl_3$) : 2.70–2.84 (m, 4H, CH_2), 3.53 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.81 (s, 3H, CH_3O), 3.84 (s, 3H, CH_3O), 6.48 (s, 1H, ArH), 6.60 (s, 1H, ArH), 7.30 (d, 2H, J = 8.8, ArH), 7.34 (d, 2H, J = 8.8, ArH). Anal. Calcd for $C_{18}H_{20}ClNO_2$: C, 68.23; H, 6.20; N, 4.32. Found: C, 68.03; H, 6.34; N, 4.41.

2-(4-Fluorobenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (7). Mp 96–98°C, yield 43%; ^{1}H NMR (δ in $CDCl_3$) : 2.77–2.87 (m, 4H, CH_2), 3.58 (s, 2H, CH_2), 3.69 (s, 2H, CH_2), 3.86 (s, 3H, CH_3O), 3.89 (s, 3H, CH_3O), 6.53 (s, 1H, ArH), 6.65 (s, 1H, ArH), 7.07–7.41 (m, 4H, ArH). Anal. Calcd for $C_{18}H_{20}FNO_2$: C, 71.87; H, 6.60; N, 4.51. Found: C, 71.74; H, 6.69; N, 4.65.

Synthesis of 1-(4-benzylpiperidin-1-yl)-2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)ethane-1,2-dione (8). A mixture of 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (**31**) (193.25 mg, 1 mmol) in DCM (3 mL), ethyl oxalylchloride (166.50 mg, 1.22 mmol) and TEA (173.70 mg, 1.72 mmol) was placed in a cylindrical quartz tube (Ø 2 cm), then stirred and irradiated in a microwave oven (280 Watt, 5 min, 25°C). The resulting mixture was diluted with DCM (15 mL), washed with a saturated $NaHCO_3$ aqueous solution (2 × 10 mL) and with water (2 × 10 mL) and then extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give the ester intermediate as a yellow oil which was dissolved in 50% EtOH/water (5 mL), basified with KOH and stirred at room temperature for 5 min. Successively the mixture was acidified by the addition of HCl (6.0*N*) and the resulting solution was extracted with chloroform (3 × 10 mL), dried over Na_2SO_4 and the solvent removed under reduced pressure to afford the corresponding

acid **32**. To a solution in DMF (5 mL) of crude material obtained in previous step (265 mg, 1 mmol) and O-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (379 mg, 1 mmol) 4-benzylpiperidine (352 mg, 2 mmol) was added; the reaction mixture was placed in a cylindrical quartz tube (Ø 2 cm), stirred and irradiated in a microwave oven for two subsequent periods in the same conditions (250 Watt, 20 min, 25°C). The cooled organic layer was diluted with water (10 mL), extracted with ethyl acetate (3 × 10 mL) and dried over Na_2SO_4. The solvent was removed until dryness under reduced pressure and the resultant crude purified by silica gel column chromatography (chloroform/methanol; 99:1) to give compound **8** as a white solid.

1-(4-Benzylpiperidin-1-yl)-2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)ethane-1,2-dione (8). Mp 115–117°C, yield 66%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 1.24–1.43 (m, 4H, CH_2), 1.62–1.74 (m, 3H, CH and CH_2), 2.53–3.04 (m, 6H, CH_2), 3.62–3.66 (m, 2H, CH_2), 3.86 (s, 6H, CH_3O), 4.69 (s, 2H, CH_2), 6.55 (s, 1H, ArH), 6.63 (s, 1H, ArH), 7.08–7.29 (m, 5H, ArH). Anal. Calcd for $C_{25}H_{30}N_2O_4$: C, 71.15; H, 7.04; N, 6.46. Found: C, 71.07; H, 7.16; N, 6.63.

Synthesis of 2-(4-benzylpiperidin-1-yl)-2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)ethanone (9). 1,2,3,4-Tetrahydro-6,7-dimethoxyisoquinoline (**31**) (193.25 mg, 1 mmol) was dissolved in DCM (10 mL) and TEA (102.20 mg, 1 mmol) was added. After the addition dropwise at 0°C of 2-chloroacetyl chloride (56 mg, 0.5 mmol), the reaction mixture was stirred at rt for 1 h. The solution was washed with water (3 × 10 mL), dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was powdered by treatment with diethyl ether to provide intermediate **33**. To a solution of 2-chloro-1-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)ethanone (**33**) (270 mg, 1 mmol) in DCM (10 mL), 4-benzylpiperidine (175.28 mg, 1 mmol) and TEA (102.20 mg, 1 mmol) were added. The mixture was heated under reflux, stirred for 24 h, cooled to r.t., washed with water (3 × 10 mL) and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was powdered by treatment with diethyl ether and crystallized from ethanol to give compound **9**.

2-Chloro-1-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl) ethanone (33). Mp 198–200°C, yield 75%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.68–2.78 (m, 2H, CH_2), 3.62–3.66 (m, 2H, CH_2), 3.70 (s, 6H, CH_3O), 4.44 (s, 2H, CH_2), 4.51 (s, 2H, CH_2), 6.74 (s, 1H, ArH), 6.79 (s, 1H, ArH). Anal. Calcd for $C_{13}H_{16}ClNO_3$: C, 57.89; H, 5.98; N, 5.19. Found: C, 57.97; H, 5.76; N, 5.23.

2-(4-Benzylpiperidin-1-yl)-2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)ethanone (9). Mp110–111°C, yield 45%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 1.25–1.32 (m, 4H, CH_2), 1.65–1.98 (m, 1H, CH), 2.01 (t, 4H, J = 10.4, CH_2N), 2.53 (d, 2H, J = 7.1, CH_2), 2.76–2.83 (m, 2H, CH_2), 3.21 (s, 2H, CH_2N), 3.77–3.81 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.64 (s, 2H, CH_2), 6.61 (s, 1H, ArH), 6.63 (s, 1H, ArH), 7.12–7.25 (m, 5H, ArH). Anal. Calcd for $C_{25}H_{32}N_2O_3$: C, 73.44; H, 7.75; N, 6.91. Found: C, 73.50; H, 7.90; N, 6.86.

General procedure for the synthesis of 2-(4-arylpiperazin-1-yl)-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl] ethanone derivatives (10-27). To a solution of the intermediate **33** (270 mg, 1 mmol) in DCM (10 mL) suitable arylpiperazine (1 mmol) and TEA (101 mg, 1 mmol) were added in the Buchi Syncore reactor. The mixture was heated under reflux and stirred for 24 h, cooled to room temperature, washed with water (10 mL × 3) and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was powdered by treatment with diethyl ether and crystallized from ethanol or, in some cases, purified by flash chromatography (FC) eluting with chloroform/ethyl acetate (90:10) as eluent to give the pure derivatives (**10**–**27**).

In order to prepare compound **10** the 4-benzylpiperazine was prepared by the following procedure: mono Boc-protected piperazine (1 mmol) was dissolved in DMF (10 mL) and treated with benzylbromide (171.04 mg, 1 mmol). The mixture was cooled at 0°C and NaH (24 mg, 1 mmol) was cautiously added. The reaction was stirred in ice-bath for 1 hour and then washed with a solution of water/diethyl ether (1:2) (3 × 8 mL). The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was powdered by treatment with diethyl ether to provide 4-benzyl-piperazine-1-carboxylic acid tert-butyl ester intermediate which was used for the next step without further purification. Deprotection was achieved by dissolving Boc-intermediate in trifluoroacetic acid. The mixture was stirred at 0°C for 30 min and then concentrated under reduced pressure. The residue was basified with saturated $NaHCO_3$ aqueous solution until pH = 9 and extracted with chloroform (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, evaporated under reduced pressure and the residue powdered by treatment with diethyl ether to give the desired 4-benzylpiperazine.

2-(4-Benzylpiperazin-1-yl)-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (10). Mp 102°C dec, yield 70%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.49–2.54 (m, 8H, CH_2), 2.82–2.86 (m, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.48–3.51 (m, 2H, CH_2), 3.76–3.81 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.86 (s, 3H, CH_3O), 4.64–4.68 (m, 2H, CH_2), 6.61 (s, 1H, ArH), 6.63 (s, 1H, ArH), 7.24–7.32 (m, 5H, ArH). Anal. Calcd for $C_{24}H_{31}N_3O_3$: C, 70.39; H, 7.63; N, 10.26. Found: C, 70.53; H, 7.43; N, 10.45.

2-[4-(4-Bromobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (11). Mp 137–139°C, yield 51%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.42–2.49 (m, 8H, CH_2), 2.64–2.76 (m, 2H, CH_2), 3.20 (s, 2H, CH_2), 3.31–3.62 (m, 4H, CH_2), 3.69 (s, 3H, CH_3O) 3.70 (s, 3H, CH_3O), 4.48–4.61 (m, 2H, CH_2), 6.71 (s, 1H, ArH), 6.75 (s, 1H, ArH), 7.20–7.53 (m, 4H, ArH). Anal. Calcd for $C_{24}H_{30}BrN_3O_3$: C, 59.02; H, 6.19; N, 8.60. Found: C, 59.17; H, 6.22; N, 8.43.

2-[4-(4-Chlorobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (12). Mp 109–111°C, yield 64%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.46–2.54 (m, 8H, CH_2), 2.74–2.86 (m, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.46 (s, 2H, CH_2), 3.75–3.82 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.64–4.68 (m, 2H, CH_2), 6.58 (s, 1H, ArH), 6.63 (s, 1H, ArH), 7.26–7.28 (m, 4H, ArH). Anal. Calcd for $C_{24}H_{30}ClN_3O_3$: C, 64.93; H, 6.81; N, 9.46. Found: C, 65.09; H, 6.96; N, 9.23.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(2-fluorobenzyl)piperazin-1-yl]ethanone (13). Mp 81–83°C, yield 70%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.52–2.74 (m, 8H, CH_2), 2.76–2.85 (m, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 3.75–3.81 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.64–4.67 (m, 2H, CH_2), 6.58 (s, 1H, ArH), 6.63 (s, 1H, ArH), 7.00–7.38 (m, 4H, ArH). Anal. Calcd for $C_{24}H_{30}FN_3O_3$: C, 67.43; H, 7.07; N, 9.83. Found: C, 67.71; H, 7.18; N, 9.29.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(3-fluorobenzyl)piperazin-1-yl]ethanone (14). Mp 88–90°C, yield 63%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.49–2.55 (m, 8H, CH_2), 2.76–2.86 (m, 2H, CH_2), 3.27 (s, 2H, CH_2), 3.47–3.49 (m, 2H, CH_2), 3.76–3.82 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.65–4.68 (m, 2H, CH_2), 6.61 (s, 1H, ArH), 6.63 (s, 1H, ArH), 6.94–7.25 (m, 4H, ArH). Anal. Calcd for $C_{24}H_{30}FN_3O_3$: C, 67.43; H, 7.07; N, 9.83. Found: C, 67.68; H, 7.11; N, 9.53.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(4-fluorobenzyl)piperazin-1-yl]ethanone (15). Mp 95–96°C, yield 72%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.34–2.47 (m, 8H, CH_2), 2.64–2.76 (m, 2H, CH_2), 3.19 (s, 2H, CH_2), 3.37–3.41 (m, 2H, CH_2), 3.58–3.62 (m, 2H, CH_2), 3.69 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 4.48–4.61 (m, 2H, CH_2), 6.72 (s, 1H, ArH), 6.75 (s, 1H, ArH), 7.09–7.32 (m, 4H, ArH). Anal. Calcd for $C_{24}H_{30}FN_3O_3$: C, 67.43; H, 7.07; N, 9.83. Found: C, 67.52; H, 7.02; N, 9.77.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(3-methylbenzylpiperazin-1-yl]ethanone (16). Mp 139–141°C, yield 55%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.34 (s, 3H, CH_3), 2.50–2.55 (m, 8H, CH_2), 2.76–2.84 (m, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.45 (s, 2H, CH_2), 3.76–3.82 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.64–4.67 (m, 2H, CH_2), 6.61 (s, 1H, ArH), 6.63 (s, 1H, ArH), 7.05–7.20 (m, 4H, ArH). Anal. Calcd for $C_{25}H_{33}N_3O_3$: C, 70.89; H, 7.85; N, 9.92. Found: C, 70.63; H, 7.71; N, 10.12.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(4-methylbenzyl)piperazin-1-yl]ethanone (17). Mp 94°C dec, yield 69%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.34 (s, 3H, CH_3), 2.46–2.54 (m, 8H, CH_2), 2.74–2.86 (m, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.46 (s, 2H, CH_2), 3.75–3.82 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.64 (s, 2H, CH_2), 6.61 (s, 1H, ArH), 6.63 (s, 1H, ArH), 7.13–7.23 (m, 4H, ArH). Anal. Calcd for $C_{25}H_{33}N_3O_3$: C, 70.89; H, 7.85; N, 9.92. Found: C, 70.73; H, 7.69; N, 9.75.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(4-methoxybenzyl)piperazin-1-yl]-ethanone (18). Mp 94°C dec, yield 69%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.46–2.53 (m, 8H, CH_2), 2.76–2.83 (m, 2H, CH_2), 3.25 (s, 2H, CH_2), 3.44 (s, 2H, CH_2), 3.71 (s, 3H, CH_3O), 3.75–3.85 (m, 5H, CH_2 and CH_3O) 3.86 (s, 3H, CH_3O), 4.64–4.68 (m, 2H, CH_2), 6.61 (s, 1H, ArH), 6.63 (s, 1H, ArH), 6.83–7.23 (m, 4H, ArH). Anal. Calcd for $C_{25}H_{33}N_3O_4$: C, 68.31; H, 7.57; N, 9.56. Found: C, 68.54; H, 7.43; N, 9.48.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(3-trifluoromethylbenzyl)piperazin-1-yl]ethanone (19). Mp 101–103°C, yield 77%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.41–2.48 (m, 8H, CH_2), 2.62–2.78 (m, 2H, CH_2), 3.20 (s, 2H, CH_2), 3.50–3.53 (m, 2H, CH_2), 3.57–3.62 (m, 2H, CH_2), 3.69 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 4.48–4.62 (m, 2H, CH_2), 6.71 (s, 1H, ArH), 6.75 (s, 1H, ArH), 7.57–7.61 (m, 4H, ArH). Anal. Calcd for $C_{25}H_{30}F_3N_3O_3$: C, 62.88; H, 6.33; N, 8.80. Found: C, 62.71; H, 6.46; N, 8.94.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(4-trifluoromethylbenzyl)piperazin-1-yl]ethanone (20). Mp 102–104°C, yield 75%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.41–2.48 (m, 8H, CH_2) 2.64–2.78 (m, 2H, CH_2), 3.19 (s, 2H, CH_2), 3.49–3.53 (m, 2H, CH_2), 3.60–3.67 (m, 2H, CH_2), 3.69 (s, 3H, CH_3O), 3.71 (s, 3H, CH_3O), 4.48–4.62 (m, 2H, CH_2), 6.71 (s, 1H, ArH), 6.75 (s, 1H, ArH), 7.48–7.68 (m, 4H, ArH). Anal. Calcd for $C_{25}H_{30}F_3N_3O_3$: C, 62.88; H, 6.33; N, 8.80. Found: C, 62.96; H, 6.52; N, 8.67.

2-[4-(4-*t*-Butylbenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (21). Mp 97–99°C, yield 80%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 1.29 (s, 9H, CH_3), 2.41–2.47 (m, 8H, CH_2), 2.70–2.81 (m, 2H, CH_2), 3.15 (s, 2H, CH_2), 3.36–3.57 (m, 4H, CH_2), 3.70 (s, 6H, CH_3O), 4.50–4.55 (m, 2H, CH_2), 6.77–6.82 (m, 2H, ArH), 7.46–7.53 (m, 4H, ArH). Anal. Calcd for $C_{28}H_{39}N_3O_3$: C, 72.23; H, 8.44; N, 9.02. Found: C, 72.47; H, 8.62; N, 9.14.

2-[4-(3,4-Dichlorobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (22). Mp 78–80°C, yield 34%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.41–2.55 (m, 8H, CH_2), 2.76–2.84 (m, 2H, CH_2), 3.27 (s, 2H, CH_2), 3.44 (s, 2H, CH_2), 3.75–3.82 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.64–4.67 (m, 2H, CH_2), 6.62 (s, 1H, ArH), 6.63 (s, 1H, ArH), 7.14–7.42 (m, 3H, ArH). Anal. Calcd for $C_{24}H_{29}Cl_2N_3O_3$: C, 60,25; H, 6.11; N, 8.78. Found: C, 60.32; H, 6.06; N, 8.90.

2-[4-(2-Chloro-6-fluorobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (23). Mp 78–80°C, yield 71%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.53–2.57 (m, 8H, CH_2), 2.74-2.86 (m, 2H, CH_2), 3.23 (s, 2H, CH_2), 3.69 (s, 2H, CH_2), 3.70-3.81 (m, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, CH_3O), 4.64-4.67 (m, 2H, CH_2), 6.61 (s, 1H, ArH), 6.63 (s, 1H, ArH), 6.95-7.20 (m, 3H, ArH). Anal. Calcd for $C_{24}H_{29}ClFN_3O_3$: C, 62.40; H, 6.33; N, 9.10. Found: C, 62.72; H, 6.48; N, 9.21.

2-[4-(2,4-Difluorobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (24). Mp 100–102°C, yield 66%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.40–2.48 (m, 8H, CH_2), 2.64–2.76 (m, 2H, CH_2), 3.19 (s, 2H, CH_2), 3.45–3.48 (m, 2H, CH_2), 3.58–3.62 (m, 2H, CH_2), 3.69 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 4.48–4.61 (m, 2H, CH_2), 6.72 (s, 1H, ArH), 6.75 (s, 1H, ArH), 7.14–7.22 (m, 3H, ArH). Anal. Calcd for $C_{24}H_{29}F_2N_3O_3$: C, 64.70; H, 6.56; N, 9.43. Found: C, 67.59; H, 6.77; N, 9.31.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(2,4,6-trimethylbenzyl)piperazin-1-yl]ethanone (25). Mp 99–101°C, yield 78%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.17 (s, 3H, CH_3), 2.26 (s, 6H, CH_3), 2.34–2.44 (m, 8H, CH_2), 2.64–2.78 (m, 2H, CH_2), 3.16 (s, 2H, CH_2), 3.36 (s, 2H, CH_2), 3.60–3.65 (m, 2H, CH_2), 3.69 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 4.62 (s, 2H, CH_2), 6.71–6.77 (m, 4H, ArH). Anal. Calcd for $C_{27}H_{37}N_3O_3$: C, 71.81; H, 8.26; N, 9.30. Found: C, 72.01; H, 8.33; N, 9.16.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-(4-phenethylpiperazin-1-yl]ethanone (26). Mp 88–90°C, yield 88%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 2.59–2.84 (m, 14H, CH_2), 3.28 (s, 2H, CH_2), 3.78–3.87 (m, 8H, CH_2 and CH_3O), 4.65–4.69 (m, 2H, CH_2), 6.58–6.62 (m, 2H, ArH), 7.26–7.28 (m, 5H, ArH). Anal. Calcd for $C_{25}H_{33}N_3O_3$: C, 70.89; H, 7.85; N, 9.92. Found: C, 70.95; H, 7.67; N, 9.81.

2-(4-*trans*-Cinnamylpiperazin-1-yl)-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (27). Mp 147–149°C, yield 73%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.41–2.48 (m, 8H, CH_2), 2.64–2.76 (m, 2H, CH_2), 3.01–3.07 (m, 2H, CH_2), 3.19 (s, 2H, CH_2), 3.35–3.60 (m, 2H, CH_2), 3.69 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 4.48–4.62 (m, 2H, CH_2), 6.23–6.53 (m, 2H, CH=CH), 6.71 (s, 1H, ArH), 6.75 (s, 1H, ArH), 7.18–7.42 (m, 5H, ArH). Anal. Calcd for $C_{25}H_{31}N_3O_3$: C, 71.70; H, 7.64; N, 9.65. Found: C, 71.83; H, 7.45; N, 9.53.

Synthesis of 2-[4-(cyclohexylmethyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone (28). Derivative **28** was obtained with the same procedure of compounds **10–27** by treatment of intermediate **33** (270 mg, 1 mmol) with 1-(cyclohexylmethyl)piperazine (183 mg, 1 mmol). Mp102–104°C, yield 80%. ^{1}H NMR (δ, ppm, in $CDCl_3$): 0.83–1.76 (m, 11H, CH_2 and CH), 2.07–2.12 (m, 2H, CH_2), 2.40–2.52 (m, 8H, CH_2), 2.75–2.86 (m, 2H, CH_2), 3.25 (s, 2H, CH_2), 3.77–3.82 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.64–4.70 (m, 2H, CH_2), 6.59–6.63 (m, 2H, ArH). Anal. Calcd for $C_{24}H_{37}N_3O_3$: C, 69.36; H, 8.97; N, 10.11. Found: C, 69.77; H, 9.05; N, 10.23.

Synthesis of 1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-((1,3-dioxolan-2-yl)methyl)piperazin-1-yl] ethanone (29). Derivative **29** was obtained with the same procedure of compounds **10–27** by treatment of intermediate **33** (270 mg, 1 mmol) with 1-[(1,3-dioxolan-2-yl)methyl]piperazine (172 mg, 1 mmol). Mp 113–115°C, yield 89%. ^{1}H nmr (δ, ppm, in $CDCl_3$): 2.58–2.60 (m, 12H, CH_2), 2.75–2.86 (m, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.76–3.80 (m, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 3.88–3.99 (m, 2H, CH_2), 4.65–4.69 (m, 2H, CH_2), 4.99–5.00 (m, 1H, CH), 6.58–6.63 (m, 2H, ArH). Anal. Calcd for $C_{21}H_{31}N_3O_5$: C, 62.20; H, 7.71; N, 10.36. Found: C, 62.41; H, 7.59; N, 10.14.

Synthesis of 2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)-1-[4-(4-methoxybenzyl)piperazin-1-yl]ethanone (30). 1-(4-Methoxybenzyl)piperazine (**34**) (206 mg, 1 mmol) was dissolved in DCM (10ml) and TEA (101 mg, 1 mmol) was added. 2-Chloroacetyl chloride (113 mg, 1 mmol) was added dropwise at 0°C and the solution stirred at room temperature for 1 h. The reaction mixture was washed with water (3 × 10 mL), dried over Na_2SO_4 and concentrated to give intermediate **35** as an oily residue, which was used in the next step without further purification. To a suspension of **35** (369 mg, 1 mmol) and NaH (120 mg, 5 mmol) in dry DMF (2 mL) **31** was added and the reaction was stirred at r.t. under N_2 atmosphere for 20 h. Then it was quenched with a saturated $NaHCO_3$ aqueous solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic phase was washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was crystallized upon trituration with diethyl ether and ethyl acetate and the solid was recrystallized from ethanol to provide compound **30**. Mp 132–133°C, yield 46%. ^{1}H NMR (δ, ppm, in DMSO-d_6): 2.25–2.29 (m, 4H, CH_2), 2.62–2.67 (m, 4H, CH_2), 3.26 (s, 2H, CH_2), 3.37 (s, 2H, CH_2), 3.43–3.52 (m, 4H, CH_2), 3.48 (s, 2H, CH_2), 3.67 (s, 3H, CH_3O), 3.69 (s, 3H, CH_3O), 3.71 (s, 3H, CH_3O), 6.59–7.18 (m, 6H, ArH). Anal. Calcd for $C_{25}H_{33}N_3O_4$: C, 68.31; H, 7.57; N, 9.56. Found: C, 68.51; H, 7.72; N, 9.39.

For the biological evaluation, the hydrochloride salts of compounds **5–7** and **9–30** were prepared as follows: the free bases were dissolved in DCM and HCl (g) was bubbled over a period of 1 h. The solvent was removed under reduced pressure to give the corresponding hydrochloride derivatives which were crystallized by diethyl ether. The obtained compounds were characterized by elemental analysis and the melting points are reported later.

2-Benzyl-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline hydrochloride (5·HCl). Mp 243–245°C. Anal. Calcd for $C_{18}H_{21}NO_2$·HCl: C, 67.60; H, 6.93; N, 4.38; Cl 11.08. Found: C, 67.72; H, 6.69; N, 4.52; Cl 11.23.

2-(4-Chlorobenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline hydrochloride (6·HCl). Mp 247–249°C. Anal. Calcd for $C_{18}H_{20}ClNO_2$·HCl: C, 61.03; H, 5.97; N, 3.95; Cl 20.01. Found: C, 61.22; H, 6.08; N, 4.11; Cl 19.89.

2-(4-Fluorobenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline hydrochloride (7·HCl). Mp 220–222°C. Anal. Calcd for $C_{18}H_{20}FNO_2$·HCl: C, 64.00; H, 6.27; N, 4.15; Cl 10.49. Found: C, 64.13; H, 6.14; N, 4.04; Cl 10.61.

2-(4-Benzylpiperidin-1-yl)-2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)ethanone hydrochloride (9·HCl). Mp 213–215°C. Anal. Calcd for $C_{25}H_{32}N_2O_3$·HCl: C, 67.48; H, 7.47; N, 6.30; Cl 7.97. Found: C, 67.29; H, 7.28; N, 6.54; Cl 8.10.

2-(4-Benzylpiperazin-1-yl)-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (10·2HCl). Mp 234–236°C. Anal. Calcd for $C_{24}H_{31}N_3O_3$·2HCl·0.5H_2O: C, 58.66; H, 6.97; N, 8.55; Cl 14.43. Found: C, 58.76; H, 7.13; N, 8.36; Cl 14.66.

2-[4-(4-Bromobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (11·2HCl). Mp 235–237°C. Anal. Calcd for $C_{24}H_{30}BrN_3O_3$·2HCl·0.5H_2O: C, 50.54; H, 5.83; N, 7.37; Cl 12.43. Found: C, 50.61; H, 5.62; N, 7.43 Cl 12.22.

2-[4-(4-Chlorobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (12·2HCl). Mp 249–251°C. Anal. Calcd for $C_{24}H_{30}ClN_3O_3$·2HCl·0.5H_2O: C, 54.81; H, 6.32; N, 7.99; Cl 20.22. Found: C, 54.69; H, 6.54; N, 8.12 Cl 20.01.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(2-fluorobenzyl)piperazin-1-yl]ethanone dihydrochloride (13·2HCl). Mp 226–228°C. Anal. Calcd for $C_{24}H_{30}FN_3O_3$·2HCl·0.5H_2O: C, 56.58; H, 6.53; N, 8.25; Cl 13.92. Found: C, 56.72; H, 6.31; N, 8.51 Cl 13.68.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(3-fluorobenzyl)piperazin-1-yl]ethanone dihydrochloride (14·2HCl). Mp 223–225°C. Anal. Calcd for $C_{24}H_{30}FN_3O_3$·2HCl·0.5H_2O: C, 56.58; H, 6.53; N, 8.25; Cl 13.92. Found: C, 56.27; H, 6.41; N, 8.52 Cl 13.73.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(4-fluorobenzyl)piperazin-1-yl]ethanone dihydrochloride (15·2HCl). Mp 230–232°C. Anal. Calcd for $C_{24}H_{30}FN_3O_3$·2HCl·0.5H_2O: C, 56.58; H, 6.53; N, 8.25; Cl 13.92. Found: C, 56.48; H, 6.72; N, 8.56 Cl 13.81.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(3-methylbenzylpiperazin-1-yl]ethanone dihydrochloride (16·2HCl). Mp 139–141°C. Anal. Calcd for $C_{25}H_{33}N_3O_3$·2HCl· 0.5H_2O: C, 59.40; H, 7.18; N, 8.31; Cl 14.03. Found: C, 59.53; H, 7.06; N, 8.52 Cl 14.28.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(4-methylbenzyl)piperazin-1-yl]ethanone dihydrochloride (17·2HCl). Mp 94°C dec. Anal. Calcd for $C_{25}H_{33}N_3O_3$·2HCl·0.5H_2O: C, 59.40; H, 7.18; N, 8.31; Cl 14.03. Found: C, 59.33; H, 6.98; N, 8.57 Cl 14.32.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(4-methoxybenzyl)piperazin-1-yl]-ethanone dihydrochloride (18·2HCl). Mp 134–136°C. Anal. Calcd for $C_{25}H_{33}N_3O_4$·2HCl·0.5H_2O: C, 57.58; H, 6.96; N, 8.06; Cl 13.60. Found: C, 57.72; H, 7.08; N, 8.24 Cl 13.41.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(3-trifluoromethylbenzyl)piperazin-1-yl]ethanone dihydrochloride (19·2HCl). Mp 230–232°C. Anal. Calcd for $C_{25}H_{30}F_3N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 53.67; H, 5.95; N, 7.51; Cl 12.67. Found: C, 53.84; H, 6.09; N, 7.38 Cl 12.52.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(4-trifluoromethylbenzyl)piperazin-1-yl]ethanone dihydrochloride (20·2HCl). Mp 222–224°C. Anal. Calcd for $C_{25}H_{30}F_3N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 53.67; H, 5.95; N, 7.51; Cl 12.67. Found: C, 53.82; H, 6.13; N, 7.72 Cl 12.49.

2-[4-(4-*t*-Butylbenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (21·2HCl). Mp 230–232°C. Anal. Calcd for $C_{28}H_{39}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 61.42; H, 7.73; N, 7.67; Cl 12.95. Found: C, 61.58; H, 7.52; N, 7.81; Cl 13.08.

2-[4-(3,4-Dichlorobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (22·2HCl). Mp 239–241°C. Anal. Calcd for $C_{24}H_{29}Cl_2N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 51.44; H, 5.76; N, 7.50; Cl 25.31. Found: C, 51.23; H, 5.42; N, 7.78; Cl 25.57.

2-[4-(2-Chloro-6-fluorobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (23·2HCl). Mp 237–239°C. Anal. Calcd for $C_{24}H_{29}ClFN_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 53.00; H, 5.93; N, 7.73; Cl 19.55. Found: C, 52.88; H, 5.87; N, 7.89; Cl 19.36.

2-[4-(2,4-Difluorobenzyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (24·2HCl). Mp 223–225°C. Anal. Calcd for $C_{24}H_{29}F_2N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 54.65; H, 6.12; N, 7.97; Cl 13.44. Found: C, 54.73; H, 6.27; N, 8.11; Cl 13.32.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-(2,4,6-trimethylbenzyl)piperazin-1-yl]ethanone dihydrochloride (25·2HCl). Mp 232–234°C. Anal. Calcd for $C_{27}H_{37}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 60.78; H, 7.56; N, 7.88; Cl 13.29. Found: C, 60.65; H, 7.43; N, 8.01; Cl 13.37.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-(4-phenethylpiperazin-1-yl]ethanone dihydrochloride (26·2HCl). Mp 246–248°C. Anal. Calcd for $C_{25}H_{33}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 59.40; H, 7.18; N, 8.31; Cl 14.03. Found: C, 59.57; H, 7.24; N, 8.17; Cl 14.12.

2-(4-*trans*-Cinnamylpiperazin-1-yl)-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (27·2HCl). Mp 249–251°C. Anal. Calcd for $C_{25}H_{31}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 59.64; H, 6.81; N, 8.35; Cl 14.08. Found: C, 59.72; H, 6.93; N, 8.22; Cl 14.16.

2-[4-Cyclohexylmethyl)piperazin-1-yl]-1-[3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]ethanone dihydrochloride (28·2HCl). Mp 232–234°C. Anal. Calcd for $C_{24}H_{37}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 57.94; H, 8.10; N, 8.45; Cl 14.25. Found: C, 58.03; H, 8.24; N, 8.70; Cl 14.12.

1-[3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl]-2-[4-((1,3-dioxolan-2-yl)methyl)piperazin-1-yl]ethanone dihydrochloride (29·2HCl). Mp 239–241°C. Anal. Calcd for $C_{21}H_{31}N_3O_5 \cdot 2HCl \cdot 0.5H_2O$: C, 51.75; H, 7.03; N, 8.62; Cl 14.55. Found: C, 51.53; H, 7.22; N, 8.54; Cl 14.23.

2-(3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)-1-[4-(4-methoxybenzyl)piperazin-1-yl]ethanone dihydrochloride (30·2HCl). Mp 249–251°C. Anal. Calcd for $C_{25}H_{33}N_3O_4 \cdot 2HCl \cdot 0.5H_2O$: C, 57.58; H, 6.96; N, 8.06; Cl 13.60. Found: C, 57.71; H, 7.13; N, 7.95; Cl 13.81.

INHIBITION OF AChE AND BChE

The cholinesterase assays were performed using colorimetric method reported by Ellman [19]. The assay solution consisted of a 0.1*M* phosphate buffer pH 8.0, with the addition of 0.01*M* 5,5′-dithio-bis(2-nitrobenzoic acid), 0.044 Unit/mL human recombinant AChE or BChE derived from human serum and 0.037*M* of substrate (acetylthiocholine iodide or butyrylthiocholine iodide, respectively). All the reagents were purchased from Sigma Chemical Company. AChE and BChE were dissolved in 0.1*M* phosphate buffer pH 8.0 containing Triton X-100 0.1%. The enzymes were diluted before use in order to reach an activity ranging between 0.250 and 0.100 AU min^{-1} in the final assay conditions. Stock solutions of the tested compounds were prepared in water. Different concentrations of each test compound (analyzed in triplicate) were used to obtain inhibition of ACh- and BChE activity ranging between 20 and 80%. Following a 20 min pre-incubation at 37°C with inhibitor and enzyme, the reaction was started by the addition of substrate. Initial rates were determined at 37°C by measuring the absorbance at 412 nm every 1 min during 5 min with a Beckman DU 800 spectrophotometer. The reaction rates were compared and the percent inhibition due to the presence of test compounds was calculated. Blanks containing all components except enzyme were run in order to account for nonenzymatic reactions. Tacrine (0.5 μ*M*), a reversible, non selective inhibitor of AChE and an ever stronger inhibitor of BChE was used to test assay efficiency (–65% and –93%, respectively). The percent inhibition of the enzyme activity due to the presence of increasing test compound concentration was calculated by the following expression: $100 - (v_i/v_0 \times 100)$, where v_i is the initial rate calculated in the presence of inhibitor and v_0 is the enzyme activity. The concentration producing 50% inhibition (IC_{50}) was calculated using a computer programme (SAS/STAT) of the method of Litchfield and Wilcoxon.

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