

3-Aryl Coumarin Derivatives Bearing Aminoalkoxy Moiety as Multi-Target-Directed Ligands against Alzheimer's Disease

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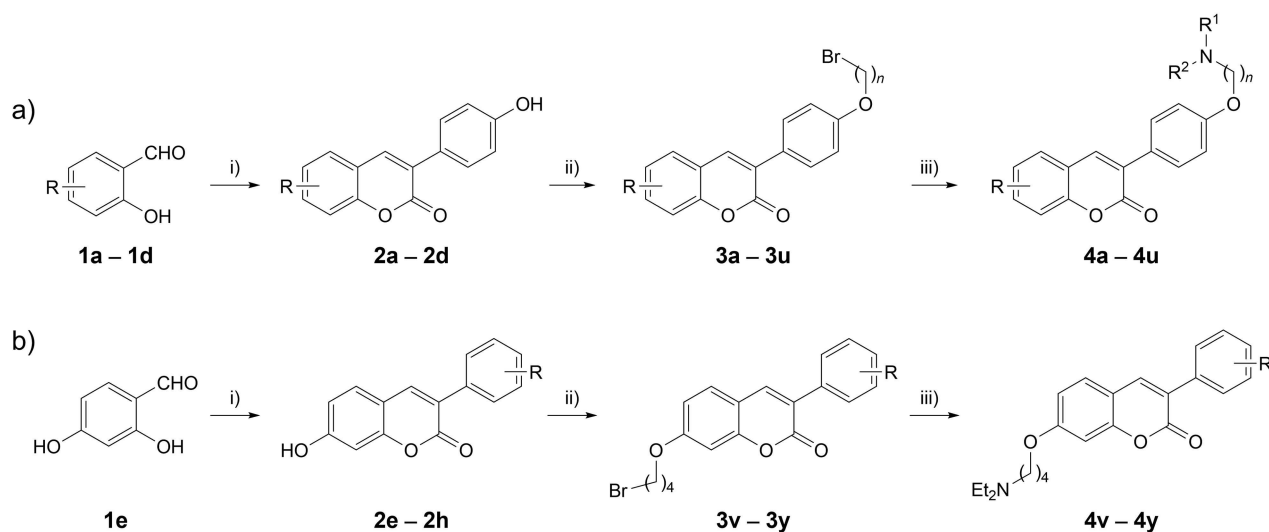
Two series of novel coumarin derivatives, substituted at 3 and 7 positions with aminoalkoxy groups, are synthesized, characterized, and screened. The effect of amine substituents and the length of cross-linker are investigated in acetyl- and butyrylcholinesterase (AChE and BuChE) inhibition. Target compounds show moderate to potent inhibitory activities against AChE and BuChE. 3-(3,4-Dichlorophenyl)-7-[4-(diethylamino) butoxy]-2H-chromen-2-one (**4y**) is identified as the most potent compound against AChE ($IC_{50} = 0.27 \mu M$). Kinetic and molecular modeling studies affirmed that compound **4y** works in a mixed-type way and interacts simultaneously with the catalytic active site (CAS) and peripheral anionic site (PAS) of AChE. In addition, compound **4y** blocks β -amyloid ($A\beta$) self-aggregation with a ratio of 44.11 % at $100 \mu M$ and significantly protects PC12 cells from H_2O_2 -damage in a dose-dependent manner.

Keywords: Alzheimer's disease, cholinesterase, neuroprotective activity, 3-phenylcoumarin, β -amyloid, synthesis design.

Introduction

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Alzheimer's disease (AD), described by Alois Alzheimer in 1907, has become the most common irreversible



Scheme 1. Synthesis of 3-aryl coumarin derivatives bearing aminoalkyl moiety (compounds **4a–4y**). Reagents and conditions: i) arylacetic acids, AcONa (dry), Ac₂O, reflux; ii) corresponding dibromoalkanes, K₂CO₃, acetone, reflux; iii) corresponding secondary amines, K₂CO₃, MeCN, reflux.

neurodegenerative and progressive disorder characterized by cognitive and memory impairment.^[1] The statistic depicts that almost 3% of elderly population aging 65–74 and 50% of 85-and-older age groups are affected by AD.^[2] The main etiology of this disease is not completely diagnosed, so, various hallmarks such as oxidative stress, inflammation, low level of acetylcholine, τ protein aggregation and amyloid- β (A β) deposits seem to play crucial roles in the disease.^[3–5] Current treatment of patients suffering from AD banks on increasing cholinergic neurotransmission in the brain through acetylcholinesterase (AChE) inhibitors like rivastigmine, donepezil, and galantamine. Despite the presence of these therapeutic agents, the complete prevention and treatment of AD still remain vague.^[6] To meet the profound need of disease-modifying drugs, new approaches have arisen in medicinal chemistry.^[7] Multipart etiology of AD propelled researchers to design structures with the ability to concurrently interact with different targets. To modulate multiple targets including acetyl- and butyrylcholinesterase (AChE and BuChE), A β aggregation along with neuroprotective properties, multi-target-directed ligands (MTDLs) working by the combination of distinct pharmacological moieties, could attain greater efficiency than that of classic drugs, based on ‘one-molecule-one-target’ strategy.^[8–10]

Coumarins are one of the privileged naturally occurring compounds which have gained fame as a key pharmacophore, showing a diverse range of biological activities including anti-inflammatory, anti-

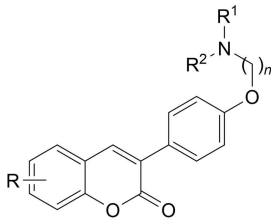
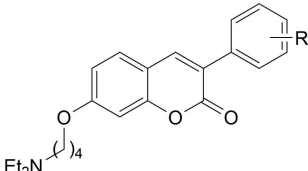
oxidant, and AChE inhibitory activities.^[11] In previous reports, coumarin derivatives exhibited their efficiency in A β aggregation together with AChE inhibitory activity.^[12,13] For example, by linking *N,N*-benzyl (methyl)amino group to 3-phenylcoumarin core, a new series of compounds were introduced, which are capable of simultaneous binding to both catalytic and peripheral anionic site of the AChE.^[14] Some of the 3-phenylcoumarin derivatives have been developed as multifunctional agents to simultaneously inhibit different enzymatic targets including monoaminoxidase (MAO), beta-secretase (BACE) and AChE for treating AD.^[15–19] In the light of the above results and in continuation of our previous works on cholinesterase inhibitors,^[20–22] herein, two series of 3-aryl coumarin derivatives bearing various secondary amine groups at 3 or 7 positions of the coumarin framework are synthesized and evaluated as AChE and BuChE inhibitors. Kinetic and ligand-enzyme docking studies, A β self and AChE-induced aggregation as well as neuroprotective activity are also investigated for the best compounds.

Results and Discussion

Chemistry

Target compounds **4a–4y** were synthesized according to the procedure illustrated in Scheme 1. Compounds **2a–2d** were synthesized by the Perkin–Oglialoro reaction between arylacetic acids and salicylaldehyde

Table 1. Inhibitory activity of synthesized compounds **4a–4y** against AChE and BuChE. Values are expressed as means $\pm$ SEM of three experiments.

Compound	<i>n</i>	R	 4a – 4u	 4v – 4y	IC ₅₀ [μ M] AChE	IC ₅₀ [μ M] BuChE
			R ¹ R ² N			
4a	3	H	Et ₂ N		8.9 $\pm$ 0.35	11.5 $\pm$ 0.46
4b	3	H	Bn(Et)N		27.2 $\pm$ 1.90	30.2 $\pm$ 2.40
4c	3	H	Bn(Me)N		26.2 $\pm$ 2.10	34.5 $\pm$ 2.40
4d	3	H	4-(2-Methoxyphenyl)piperazin-1-yl		N.A.	N.A.
4e	4	H	Bu ₂ N		3.12 $\pm$ 0.25	2.0 $\pm$ 0.12
4f	4	H	Bn(Et)N		2.7 $\pm$ 0.13	2.6 $\pm$ 0.15
4g	4	H	Bn(Me)N		4.3 $\pm$ 0.34	5.9 $\pm$ 0.23
4h	4	H	Pyrrolidin-1-yl		1.16 $\pm$ 0.08	2.5 $\pm$ 0.33
4i	4	H	Piperidin-1-yl		1.86 $\pm$ 0.11	2.26 $\pm$ 0.21
4j	4	H	4-(2-Methoxyphenyl)piperazin-1-yl		N.A. ^a	N.A.
4k	4	H	Et ₂ N		1.6 $\pm$ 0.10	2.4 $\pm$ 0.17
4l	4	7-Br	Et ₂ N		0.95 $\pm$ 0.01	83.6 $\pm$ 3.20
4m	4	8-MeO	Et ₂ N		1.3 $\pm$ 0.10	2.8 $\pm$ 0.14
4n	4	5,6-C ₄ H ₄	Et ₂ N		0.5 $\pm$ 0.01	2.67 $\pm$ 0.61
4o	5	H	Et ₂ N		5.8 $\pm$ 0.35	5.4 $\pm$ 0.38
4p	5	H	Bn(Et)N		19.1 $\pm$ 1.10	29.4 $\pm$ 1.40
4q	5	H	Bn(Me)N		20.2 $\pm$ 1.40	30.7 $\pm$ 1.50
4r	5	H	Piperidin-1-yl		2.5 $\pm$ 0.12	2.8 $\pm$ 0.19
4s	5	H	Morpholin-4-yl		N.A.	N.A.
4t	5	H	4-(2-Methoxyphenyl)piperazin-1-yl		N.A.	N.A.
4u	5	5,6-C ₄ H ₄	Et ₂ N		1.2 $\pm$ 0.41	2.64 $\pm$ 0.32
4v	4	H	Et ₂ N		0.53 $\pm$ 0.01	14.7 $\pm$ 1.10
4w	4	4-Br	Et ₂ N		0.31 $\pm$ 0.01	10.37 $\pm$ 1.81
4x	4	4-MeO	Et ₂ N		0.53 $\pm$ 0.01	11.3 $\pm$ 2.22
4y	4	3,4-Cl ₂	Et ₂ N		0.27 $\pm$ 0.01	10.1 $\pm$ 1.81
Donepezil					0.03 $\pm$ 0.01	4.8 $\pm$ 0.19

^a Not active. Data are expressed as the mean $\pm$ SEM of three independent replicates.

derivatives in acetic anhydride. The intermediates **3a–3u** were prepared by the reaction of compounds **2a–2d** and excessive amounts of dibromoalkanes ($n = 3–5$) in acetone under reflux condition and in the presence of K₂CO₃ as a base. The latter was finally reacted with the commercially available secondary amines in the presence of anhydrous K₂CO₃ which provided the target compounds **4a–4y** in yields of 72–91 %.

Pharmacology

Cholinesterase Inhibitory Activity. The potency of the compounds against AChE and BuChE was assessed by

the Ellman's method. The IC₅₀ values are summarized in Table 1. All tested compounds (**4a–4y**) exhibited AChE and BuChE inhibitory activities in micromolar ranges. Donepezil was utilized as a positive control. As implied from Table 1, the activity of compounds is strongly attributed to the length of the cross-linker. Having the same amino alkyl substituent, the four atom chain length is optimum for the best AChE inhibitory activity. Extending or shortening the linker led to the decreased activities. The similar trend was also observed in the case of BuChE. Comparing the IC₅₀ values of compounds with same amine groups revealed that the order of the activity is 4 > 5 > 3, based on the carbon chain length. Considering the

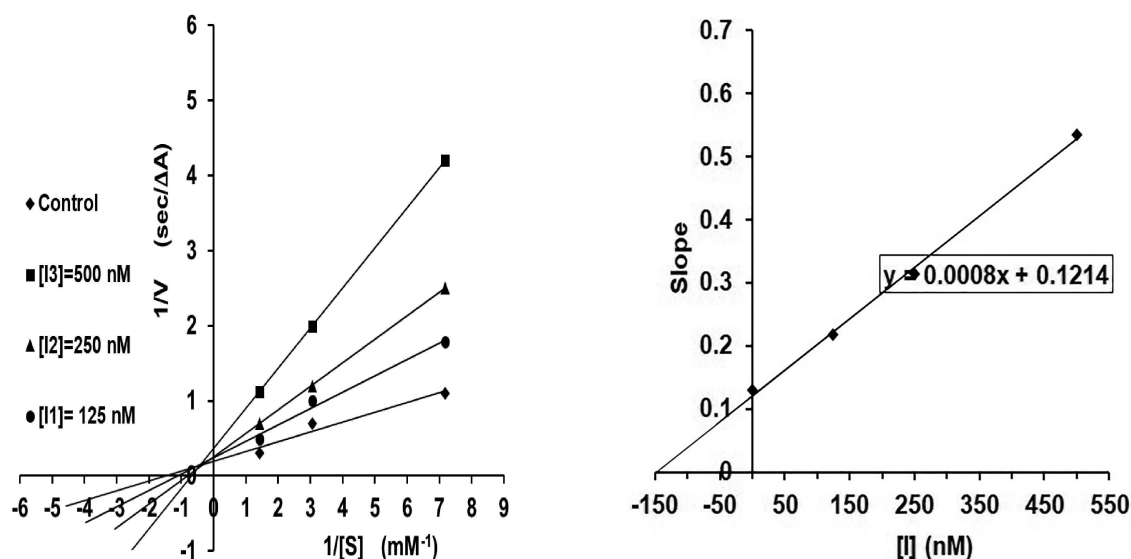


Figure 1. Lineweaver–Burk plot for the inhibition of AChE by compound **4y** at different concentrations of substrate (ATCh; left). Secondary plot of the compound for AChE K_i calculation (right).

IC_{50} values with same linker length revealed that by increasing the bulkiness of amine moiety the activity was dropped off. The flat and aromatic characters of coumarin nucleus provide a suitable condition for the pi-pi stacking interaction. Based on this hypothesis, benzo[*f*]coumarin derivative exhibited improved activity due to extra pi-pi stacking with the receptor (**4n**, $IC_{50} = 0.5 \mu\text{M}$).

With these results in hand, 7-position-modified derivatives of coumarin bearing diethylamino group *via* four carbon chain length were prepared (**4v–4y**) and evaluated against AChE and BuChE. The results showed that the activity of the 7-aminoalkoxy derivatives (**4v–4y**) against AChE was better than the 3-position-modified derivatives. Among the 7-aminoalkoxy congeners, compound **4y** showed superior activity towards AChE ($IC_{50} = 0.27 \mu\text{M}$). This could be possibly due to the contribution of the phenyl ring at 3 position of coumarin framework in the hydrophobic interaction with the enzyme's active site.

Kinetic Study of AChE Inhibition

The kinetic characterization of the most active compound **4y** against AChE was also studied. Reciprocal Lineweaver-Burk plots of $1/\text{velocity}$ vs. $1/[\text{substrate}]$ are depicted at different concentrations of the substrate (500, 250, and 125 nM). Enzyme kinetic assay on AChE was performed using compound **4y** to elucidate the inhibition type and inhibition constant. The analysis was carried out by measuring enzyme's

activity at different concentrations of substrate. The acquired inhibition data were further analyzed with Lineweaver-Burk method followed by dissociating constant (K_i) calculation using Lineweaver-Burk secondary plot. Based on the results, the mixed type of inhibition on AChE with K_i value of $0.149 \mu\text{M}$ was obtained. The dual mode of inhibition was observed for compound **4y** (competitive and uncompetitive) which is in agreement with the docking study showing simultaneously binding of compound **4y** to both catalytic active site (CAS) and peripheral anionic site (PAS) of the enzyme. The Lineweaver–Burk double reciprocal plots and secondary plot of compound **4y** are denoted in Figure 1.

Neuroprotective Activity of Compounds **4w** and **4y** against H_2O_2 -Induced Cell Death in PC12 Cells

It is well established that the protection against oxidative stress, generated by free radicals, is one of the most useful approaches in the management of AD.^[23] Compounds **4w** and **4y** with the highest anti-AChE activity were selected for evaluating their neuroprotective potential against H_2O_2 -induced PC12 cell injury at the concentrations of 0.1, 1, 5, 10, and $20 \mu\text{M}$ *via* MTT-based colorimetric assay. The cell viabilities are listed in Table 2. All compounds significantly increased PC12 cell viability in the presence of H_2O_2 . Compound **4w** showed the best neuroprotection against H_2O_2 comparable to quercetin as standard control.

Table 2. The protective effect of selected compounds against H₂O₂ (150 μ M) induced injury in PC12 cell line at different concentrations.^[a]

Compound	0.1 μ M	1 μ M	5 μ M	10 μ M	20 μ M	H ₂ O ₂
4w	50.2 $\pm$ 3.2**	55.7 $\pm$ 2.3**	69.8 $\pm$ 2.9**	77.1 $\pm$ 1.7**	82.0 $\pm$ 1.8**	29.52 $\pm$ 1.2
4y	44.5 $\pm$ 0.8**	46.5 $\pm$ 2.1**	54.2 $\pm$ 0.9**	62.9 $\pm$ 0.8**	68.6 $\pm$ 1.3**	33.8 $\pm$ 1.4
Quercetin	36.2 $\pm$ 2.3**	40.7 $\pm$ 1.4**	47.3 $\pm$ 0.8**	53.7 $\pm$ 1.2**	56.9 $\pm$ 1.6**	28.6 $\pm$ 0.9

^[a] PC12 cells viability (% of control). Data are expressed as the mean $\pm$ SEM of three independent replicates. ** $p \leq 0.001$ and not significant (ns) values with respect to the control group were obtained after one-way ANOVA analysis followed by Newman–Keuls post hoc test.

Inhibitory Effects on A β 1–42 Self and AChE-Induced Aggregation

Among the target compounds, the A β anti-aggregating properties of the most active AChE inhibitors were determined using thioflavin T (ThT) fluorescence assay and compared with donepezil and rifampicin as reference compounds. Results in Table 3 showed that compounds **4y** and **4w** are active to inhibit A β self-aggregation. Compounds **4y** and **4w** showed good potency to inhibit the A β self-aggregation (44.11 and 43.89% inhibition at 100 μ M concentration, respectively) which is two times more than that of donepezil (22% at 100 μ M concentration).

Ligand-Protein Docking Study

To elucidate the interaction mode of compound **4y** and AChE, the docking study was performed using Autodock package. The structure of AChE complex with donepezil (PDB code: 1EVE) was used as a model for docking. Compound **4y** located along the gorge of the enzyme and occupied both CAS and PAS (Figure 2). The aminoalkoxy moiety was involved in hydrophobic

interactions with CAS residues including TRP83, His439, and Phe289. The coumarin ring was also stacked against Tyr333 and the phenyl ring made a pi-pi stacking with Trp278 (Figure 2).

Conclusions

Drug discovery in AD is turning from modulation of a single target to the ‘multi-target-directed ligands’ (MTDLs) strategy. In this study, 25 new coumarin-O-alkylamine derivatives have been synthesized and evaluated as novel multifunctional agents against AD. Most of the compounds were potent in inhibition of both AChE and BuChE. Compound **4y** was the most potent derivative for AChE inhibition (IC₅₀ = 0.27 μ M) and compound **4e** showed the highest inhibitory activity against BuChE (IC₅₀ = 2.0 μ M). The kinetic analysis and docking study suggested mixed-type inhibition and binding to both CAS and PAS of AChE for compound **4y**. Importantly, compound **4y** showed effective inhibitory activity against A β -self aggregation and significant neuroprotective activity against oxidative stress H₂O₂-induced in PC12 cells compared to the appropriate standard control. All of the results highlight compound **4y** as a candidate for further studies for the development of novel MTDLs against AD.

Experimental Section

Chemistry

The progress of the reaction was monitored by TLC (silica gel 250 micron, F₂₅₄ plates, Merck). Melting points were measured on a Kofler hot stage apparatus. IR spectra were taken by PerkinElmer Spectrum version 10.03.06 (KBr disks); $\tilde{\nu}$ in cm^{−1}. ¹H-NMR spectra were recorded on a Bruker 500 MHz instrument; δ in ppm relative to Me₄Si as internal standard, J in Hz. Scheme 1 shows the atoms numbering of the final compounds

Table 3. Inhibition of AChE-induced and self-induced A β aggregation, induced by compounds **4w** and **4y**.^[a]

Compound	Self-induced ^[a]	AChE-induced ^[b]
4w	43.89 $\pm$ 0.7	12.14 $\pm$ 7.3
4y	44.11 $\pm$ 1.0	6.65 $\pm$ 8.9
Rifampicin	27.5 $\pm$ 4.3	12.2 $\pm$ 3.0
Donepezil	22.0 $\pm$ 5.4	26.1 $\pm$ 2.5

^[a] Inhibition of self-induced A β (1–42) aggregation (10 μ M) produced by the tested compound at 100 μ M concentration. Values are expressed as means $\pm$ SEM of three experiments.

^[b] Co-aggregation inhibition of A β (1–42) and AChE (0.01 units/ml) by the tested compound at 100 μ M concentration was detected by ThT assay. Values are expressed as means $\pm$ SEM of three experiments.

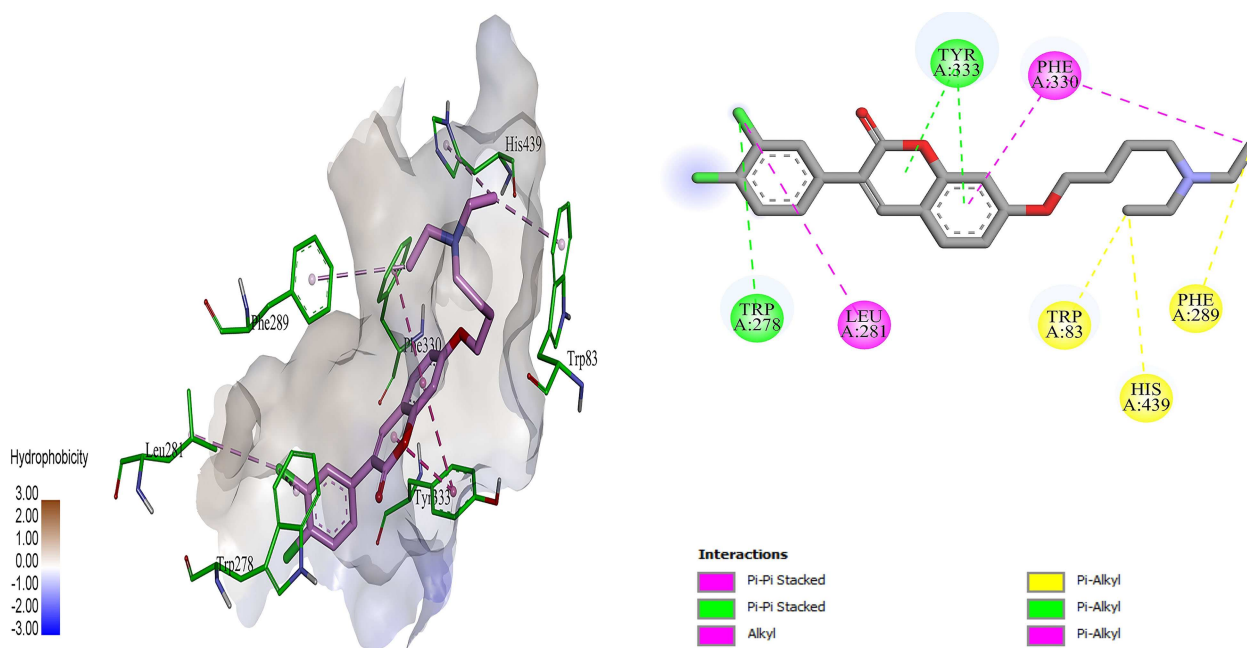


Figure 2. The proposed orientation of compound **4y** in the AChE active site (left). Illustration of compound **4y** binding mode in the AChE active site (right). The hydrophobic interactions are presented as yellow dashed lines and rings having π - π stacking are with dashed green color.

4a–4y applied in the $^1\text{H-NMR}$ interpretation. Elemental analyses were performed with CHN-Rapid Heraeus Elemental Analyzer. The results of the elemental analyses (C, H, N) were within $\pm 0.5\%$ of the calculated values.

General Procedure for the Preparation of 3-Phenyl Coumarin Derivatives (**2a–2h**)

Compounds **2a–2h** were prepared according to the previously reported method.^[24] A solution containing anhydrous AcONa (2 mmol), desired arylacetic acid (1 mmol), and appropriate salicylaldehyde (1 mmol) in Ac_2O (1 mL) was refluxed for 2 h. After completion of the reaction, the mixture was cooled, neutralized with an aqueous NaHCO_3 solution, filtered, and washed with AcOEt. The compound was used in next step without further purification.

General Procedure for the Preparation of Intermediates (**3a–3u**)

Preparation of compounds **3** was carried out according to the previously reported method.^[25] Briefly, to a solution of 3-aryl coumarin (1 mmol) and anhydrous K_2CO_3 (2 mmol) in acetone (3 mL), corresponding dibromoalkane (10 mmol) was added and refluxed for

4 h until the starting material disappeared (monitored by TLC). The solvent was removed under vacuum, hexane was added to the residue, and the product was filtered off and used in next step without further purification.

General Procedure for the Preparation of Compounds (**4a–4y**)

To a solution containing compound **3** (1 mmol) in MeCN (5 mL), appropriate secondary amines (2 mmol) and anhydrous K_2CO_3 (4 mmol) were added. The mixture was stirred under reflux condition for 24 h. After this time, the solvent was removed under vacuum, and the target product was purified by recrystallization from hexane (See the *Supporting Information* for detailed analytical data).

3-[4-[3-(Diethylamino)propoxy]phenyl]-2H-chromen-2-one (4a). Yield: 77%. White solid. M.p. 131–133 °C. IR (KBr): 2990–2750 (CH aliphatic), 1719 (C=O). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): 7.76 (s, $\text{H}_{4\text{cou}}$, 1H), 7.66 (d, $J=8.0$, $\text{H}_{2',6'}$, 2H), 7.54–7.49 (m, $\text{H}_{5,7\text{cou}}$, 2H), 7.35 (d, $J=8.0$, $\text{H}_{8\text{cou}}$, 1H), 7.29 (t, $J=7.0$, $\text{H}_{6\text{cou}}$, 1H), 6.95 (d, $J=8.0$, $\text{H}_{3',5'}$, 2H), 4.08 (t, $J=6.0$, OCH_2 , 2H), 2.91–2.81 (m, 3 NCH_2 , 6H), 2.13–2.11 (m, CH_2 , 2H), 1.20 (t, $J=7.0$, 2 Me, 6H). $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz): 161.3, 159.2,

153.5, 138.5, 131.0, 129.8, 127.6, 127.3, 124.4, 119.8, 116.3, 114.4, 65.8, 49.2, 46.7, 25.5, 10.2. Anal. calc. for $C_{22}H_{25}NO_3$: C, 75.19; H, 7.17; N, 3.99. Found: C, 75.32; H, 7.45; N, 4.25.

3-(4-{3-[Benzyl(ethyl)amino]propoxy}phenyl)-2H-chromen-2-one (4b). Yield: 77%. White solid. M.p. 58–60 °C. IR (KBr): 2960–2800 (CH aliphatic), 1717 (C=O). 1H -NMR ($CDCl_3$, 500 MHz): 7.75 (s, $H_{4_{cou.}}$, 1H), 7.65 (d, $J=8.0$, $H_{2',6'}$, 2H), 7.52–7.48 (m, $H_{5,7_{cou.}}$, 2H), 7.36–7.20 (m, 5H phenyl and $H_{6,8_{cou.}}$, 7H), 6.92 (d, $J=8.5$, $H_{3',5'}$, 2H), 4.04 (t, $J=6.0$, OCH_2 , 2H), 3.59 (s, NCH_2 -phenyl, 2H), 2.62 (t, $J=7.0$, NCH_2 , 2H), 2.54 (q, $J=7.0$, NCH_2Me , 2H), 1.97–1.92 (m, CH_2 , 2H), 1.05 (t, $J=7.0$, Me, 3H). ^{13}C -NMR ($CDCl_3$, 125 MHz): 160.7, 159.7, 153.3, 148.5, 144.2, 139.9, 138.3, 130.9, 129.7, 128.7, 128.1, 128.0, 127.6, 126.8, 126.7, 124.3, 119.9, 116.3, 114.4, 66.2, 58.2, 49.5, 47.4, 27.0, 11.8. Anal. calc. for $C_{27}H_{27}NO_3$: C, 78.42; H, 6.58; N, 3.39. Found: C, 78.58; H, 6.62; N, 3.62.

3-(4-{3-[Benzyl(methyl)amino]propoxy}phenyl)-2H-chromen-2-one (4c). Yield: 83%. White solid. M.p. 70–72 °C. IR (KBr): 2958–2775 (CH aliphatic), 1720 (C=O). 1H -NMR ($CDCl_3$, 500 MHz): 7.75 (s, $H_{4_{cou.}}$, 1H), 7.65 (d, $J=8.0$, $H_{2',6'}$, 2H), 7.53–7.48 (m, $H_{5,7_{cou.}}$, 2H), 7.36–7.25 (m, 5H phenyl and $H_{6,8_{cou.}}$, 7H), 6.94 (d, $J=8.0$, $H_{3',5'}$, 2H), 4.07 (t, $J=6.0$, OCH_2 , 2H), 3.52 (s, NCH_2 -phenyl, 2H), 2.57 (t, $J=7.0$, NCH_2 , 2H), 2.23 (s, Me, 3H), 2.03–1.99 (m, CH_2 , 2H). ^{13}C -NMR ($CDCl_3$, 125 MHz): 160.7, 159.7, 153.3, 139.0, 138.3, 130.9, 129.7, 128.9, 128.2, 127.9, 127.6, 126.9, 124.4, 119.8, 116.3, 114.5, 66.2, 62.4, 53.7, 42.2, 27.2. Anal. calc. for $C_{26}H_{25}NO_3$: C, 78.17; H, 6.31; N, 3.51. Found: C, 78.31; H, 6.15; N, 3.45.

3-(4-{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propoxy}phenyl)-2H-chromen-2-one (4d). Yield: 76%. White solid. M.p. 140–142 °C. IR (KBr): 2948–2813 (CH aliphatic), 1721 (C=O). 1H -NMR ($CDCl_3$, 500 MHz): 7.76 (s, $H_{4_{cou.}}$, 1H), 7.66 (d, $J=8.0$, $H_{2',6'}$, 2H), 7.53–7.50 (m, $H_{5,7_{cou.}}$, 2H), 7.35 (d, $J=8.0$, $H_{6_{cou.}}$, 1H), 7.29–7.25 (m, $H_{8_{cou.}}$, 1H), 6.99–6.85 (m, 4H phenyl and $H_{3',5'}$, 6H), 4.10 (br. s, OCH_2 , 2H), 3.86 (s, MeO, 3H), 3.11 (br. s, piperazine, 4H), 2.69 (br. s, piperazine, 4H), 2.04 (br. s, NCH_2 , 2H), 1.59 (br. s, CH_2 , 2H). ^{13}C -NMR ($CDCl_3$, 125 MHz): 161.1, 159.6, 153.3, 138.3, 130.9, 129.8, 127.6, 124.4, 122.8, 121.0, 118.2, 116.4, 114.5, 111.2, 66.4, 55.3, 55.1, 53.5, 50.6, 26.7. Anal. calc. for $C_{29}H_{30}N_2O_4$: C, 74.02; H, 6.43; N, 5.95. Found: C, 74.47; H, 6.28; N, 6.31.

3-{4-[4-(Dibutylamino)butoxy]phenyl}-2H-chromen-2-one (4e). Yield: 80%. White solid. M.p. 61–63 °C. IR (KBr): 2953–2875 (CH aliphatic), 1712 (C=O). 1H -NMR ($CDCl_3$, 500 MHz): 7.76 (s, $H_{4_{cou.}}$, 1H), 7.65 (d, $J=8.0$, $H_{2',6'}$, 2H), 7.53–7.48 (m, $H_{5,7_{cou.}}$, 2H), 7.36–7.26 (m, $H_{6,8_{cou.}}$, 2H), 6.95 (d, $J=8.0$, $H_{3',5'}$, 2H), 4.02 (t, $J=5.7$, OCH_2 , 2H), 2.50–2.41 (m, 3 NCH_2 , 6H), 1.82–1.79 (m, CH_2 , 2H), 1.64–1.63 (m, CH_2 , 2H), 1.44–1.40 (m, 2 CH_2 , 4H), 1.32–1.28 (m, 2 CH_2 , 4H), 0.91 (t, $J=7.0$, 2 Me, 6H). ^{13}C -NMR ($CDCl_3$, 125 MHz): 160.7, 159.6, 153.1, 138.3, 130.9, 129.7, 127.8, 127.6, 126.7, 124.3, 119.7, 116.3, 114.3, 67.8, 53.7, 53.6, 29.0, 27.1, 23.4, 20.7, 14.0. Anal. calc. for $C_{27}H_{35}NO_3$: C, 76.92; H, 8.37; N, 3.32. Found: C, 76.95; H, 8.41; N, 3.36.

3-(4-{4-[Benzyl(ethyl)amino]butoxy}phenyl)-2H-chromen-2-one (4f). Yield: 84%. White solid. M.p. 77–78 °C. IR (KBr): 3077–3078 (CH aliphatic), 1716 (C=O). 1H -NMR ($CDCl_3$, 500 MHz): 7.74 (s, $H_{4_{cou.}}$, 1H), 7.64 (d, $J=8.0$, $H_{2',6'}$, 2H), 7.52–7.48 (m, $H_{5,7_{cou.}}$, 2H), 7.35–7.22 (m, 5H phenyl and $H_{6,8_{cou.}}$, 7H), 6.92 (d, $J=8.0$, $H_{3',5'}$, 2H), 3.96 (t, $J=6.0$, OCH_2 , 2H), 3.57 (s, NCH_2 -phenyl, 2H), 2.53–2.48 (m, 2 NCH_2 , 4H), 1.81–1.80 (m, CH_2 , 2H), 1.66–1.65 (m, CH_2 , 2H), 1.04 (t, $J=7.0$, Me, 3H). ^{13}C -NMR ($CDCl_3$, 125 MHz): 160.7, 159.7, 153.3, 140.0, 138.3, 130.9, 129.7, 128.8, 128.1, 127.6, 126.6, 124.3, 119.8, 116.3, 114.4, 67.8, 58.1, 52.6, 47.2, 27.0, 23.5, 11.8. Anal. calc. for $C_{28}H_{29}NO_3$: C, 78.66; H, 6.84; N, 3.28. Found: C, 78.89; H, 6.57; N, 3.64.

Pharmacology

In Vitro AChE/BuChE Inhibition Assay. The inhibitory activity was determined by Ellman's method.^[26] AChE (electric eel, Type V–S, EC 3.1.1.7), BuChE (equine serum, EC 3.1.1.8), 5,5'-dithiobis(2-nitrobenzoic acid), acetylthiocholine (ATC) and butyrylthiocholine (BTC) iodides were attained from Sigma-Aldrich. Enzyme solutions AChE/BuChE were prepared to give 0.2 units/mL (phosphate-pH 8.0 containing 25 % v/v glycerol) in 10 mL aliquots. DTNB and ATC/BTC iodide solutions were prepared in distilled water. A solution of the corresponding compounds in EtOH/DMSO (9:1) were prepared and diluted to the final concentration (at least five concentrations) to achieve inhibition in the range of 20 to 80 percent. For assay, 20 μ L of the corresponding enzyme was added in a 24-well plate containing 2000 μ L of PBS and 30 μ L of the test compound solution, incubated for 5 min at 25 °C. Then, 60 μ L of DTNB were added. To start the reaction, 20 μ L of the substrate solution (ATC/BTC) was added and then incubated for at least 1 min at 25 °C. The

absorbance of each well was recorded at 412 nm using a microplate reader (BioTek synergy HT). For the reference value, 30 μ L of the solvent were replaced with the test compound solution. Results are expressed as the mean $\pm$ SD at least three different experiments. The inhibition curve was obtained by plotting percentage of enzyme activity (100% for the reference) vs. logarithm of the test compound concentration.

Neuroprotection Assay against H₂O₂-Induced Cell Death in PC12 Cells

Cell viability of rat differentiated PC12 cells (provided by the Iranian Biological Resource Center, IBRC, Tehran, Iran) was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay according to the previously reported method.^[27] Cells were seeded in 96-well microplates (10,000 cells per well) and incubated at 37 °C in a CO₂ incubator (5% CO₂) for 24 h in order to adhere the cells to the well bottom. Then, cells were treated with different concentrations (0.1, 1, 5, 10, and 20 μ M) of each compound and quercetin for 3 h before being exposed to H₂O₂ (150 μ M) for 2 h. MTT solution (20 μ L, 5 mg/mL) was added after the removal of the previous medium and incubated in a CO₂ incubator for 4 h. Afterward, the medium was removed and DMSO (100 μ L) was added into each well for dissolving the precipitated formed formazan crystals. Finally, the optical density (OD) was determined at 570 nm using the Synergy 2 multi-mode plate reader (Biotek, Winooski, VT, USA). Cell viability was then calculated in comparison to the untreated well. All above mentioned experiments were performed in triplicates and mean $\pm$ SEM of the obtained results were reported.

Determination of the Inhibitory Potency on A β 1–42 Self and AChE-Induced Aggregation

Inhibitory properties of the compounds on self-induced and AChE induced aggregation of amyloid- β protein 1–42 was determined using a thioflavin T (ThT)-based fluorescence assay.^[28] The ThT excitation/emission was measured at 448 nm/490 nm after 48 h using a Microplate Reader (SpectraMax). Amyloid β protein 1–42 (Sigma A9810) was dissolved in Phosphate Buffer Saline (PBS, pH = 7.4, HyClone Thermo Scientific) containing 1% ammonium hydroxide. 50 μ M Amyloid β protein 1–42 was incubated for 72 h at 37 °C for prefibrillation.

To determine the potency of the compounds in inhibition of AChE-induced aggregation, A β 1–42 (10 μ L) $\pm$ human recombinant AChE (0.01 units/ml, Sigma C1682) were added to 0.05 M KP buffer (pH 7.4) and incubated at 37 °C for 48 h in the absence and presence of test compounds (100 μ M). The incubated mixture (100 μ L) was mixed with 50 μ L of thioflavin T (ThT, 200 μ M) in 50 mM glycine-NaOH buffer (pH 8.5). Rifampicin (100 μ M, Sigma R-3501) and donepezil (100 μ M, Sigma D-6821) were also tested as reference agents. Self or AChE-induced aggregation in the presence of the tested compounds were determined by the following calculation: $(IF_i/IF_o) \times 100$, where IF_i and IF_o are the fluorescence intensities obtained for A β $\pm$ AChE in the presence and in the absence of inhibitors.

Docking Simulations

Docking simulations were performed using the Auto-dock Vina program.^[29] The crystal structure of AChE complexed with donepezil (1EVE) was obtained from Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>). After optimization of the receptor and structure of the ligands, they were converted to pdbqt format using Autodock Tools.^[30] The active site (box center and size) was set as 15 $\times$ 15 $\times$ 15 Å, the center of grid box was placed at x = 2.023, y = 63.295, z = 67.062. The exhaustiveness was 100, and the remaining parameters were accepted as default. The lowest energy conformation of the highest populated cluster was selected for further analysis. The molecular visualization was carried out in Discovery Studio 2.0 software.

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Author Contribution Statement

H. A., M. G., I. S. K., and L. J. B. performed synthetic part. H. N., H. F. and T. T. K. performed biological studies. Z. A., A. A., and B. A. coordinated in the biological tests. S. E. S. E., S. M., A. F., E. H., and M. K. supervised the project, analyzed the data, and wrote the manuscript.

References

- [1] E. Scarpini, P. Scheltens, H. Feldman, 'Treatment of Alzheimer's disease: current status and new perspectives', *Lancet Neurol.* **2003**, 2, 539–547.
- [2] M. Prince, A. Wimo, M. Guerchet, G. C. Ali, Y. T. Wu, M. Prina, 'Alzheimer's Disease International', King's College, London, UK, 2015.
- [3] Z. Wang, Y. Wang, W. Li, F. Mao, Y. Sun, L. Huang, X. Li, 'Design, Synthesis, and Evaluation of Multitarget-Directed Selenium-Containing Clioquinol Derivatives for the Treatment of Alzheimer's Disease', *ACS Chem. Neurosci.* **2014**, 5, 952–962.
- [4] M. G. Savelieff, S. Lee, Y. Liu, M. H. Lim, 'Untangling amyloid- β , tau, and metals in Alzheimer's disease', *ACS Chem. Biol.* **2013**, 8, 856–865.
- [5] S. S. Choi, S.-R. Lee, S. U. Kim, H. J. Lee, 'Alzheimer's Disease and Stem Cell Therapy', *Exp. Neurol.* **2014**, 23, 45–52.
- [6] C. Rochais, C. Lecoutey, F. Gaven, P. Giannoni, K. Hamidouche, D. Hedou, E. Dubost, D. Genest, S. Yahiaoui, T. Freret, V. Bouet, F. Dauphin, J. S. de Oliveira Santos, C. Ballandonne, S. Corvaisier, A. Malzert-Fréon, R. Legay, M. Boulouard, S. Claeysen, P. Dallemagne, 'Novel Multitarget-Directed Ligands (MTDL with Acetylcholinesterase (AChE) Inhibitory and Serotonergic Subtype 4 Receptor (5-HT₄R) Agonist Activities As Potential Agents against Alzheimer's Disease: The Design of Donecopride', *J. Med. Chem.* **2015**, 58, 3172–3187.
- [7] M. Citron, 'Alzheimer's disease: strategies for disease modification', *Nat. Rev. Drug Discovery* **2010**, 9, 387–398.
- [8] C. Zhang, Q.-Y. Du, L.-D. Chen, W.-H. Wu, S.-Y. Liao, L.-H. Yu, X.-T. Liang, 'Design, synthesis and evaluation of novel tacrine-multialkoxymethylene hybrids as multi-targeted compounds against Alzheimer's disease', *Eur. J. Med. Chem.* **2016**, 116, 200–209.
- [9] A. Cavalli, M. L. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, M. Recanatini, C. Melchiorre, 'Multi-Target-Directed Ligands to Combat Neurodegenerative Diseases', *J. Med. Chem.* **2008**, 51, 347–372.
- [10] A. Agis-Torres, M. Sollhuber, M. Fernandez, J. M. Sanchez-Montero, 'Multi-Target-Directed Ligands and Other Therapeutic Strategies in the Search of a Real Solution for Alzheimer's Disease', *Curr. Neuropharmacol.* **2014**, 12, 2–36.
- [11] X.-M. Peng, G. L. V. Damu, C.-H. Zhou, 'Current developments of coumarin compounds in medicinal chemistry', *Curr. Pharm. Des.* **2013**, 19, 3884–3930.
- [12] L. Piazza, A. Rampa, A. Bisi, S. Gobbi, F. Belluti, A. Cavalli, M. Bartolini, V. Andrisano, P. Valenti, M. Recanatini, '3-(4-[(Benzyl(methyl)amino)methyl]phenyl)-6,7-dimethoxy-2H-2-chromenone (AP2238) Inhibits Both Acetylcholinesterase and Acetylcholinesterase-Induced β -Amyloid Aggregation: A Dual Function Lead for Alzheimer's Disease Therapy', *J. Med. Chem.* **2003**, 46, 2279–2282.
- [13] P. Anand, B. Singh, N. Singh, 'A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease', *Bioorg. Med. Chem.* **2012**, 20, 1175–1180.
- [14] L. Piazza, A. Cavalli, F. Belluti, A. Bisi, S. Gobbi, S. Rizzo, M. Bartolini, V. Andrisano, M. Recanatini, A. Rampa, 'Extensive SAR and Computational Studies of 3-(4-[(Benzylmethylamino)methyl]phenyl)-6,7-dimethoxy-2H-2-chromenone (AP2238) Derivatives', *J. Med. Chem.* **2007**, 50, 4250–4254.
- [15] M. J. Matos, D. Viña, C. Picciau, F. Orallo, L. Santana, E. Uriarte, 'Synthesis and evaluation of 6-methyl-3-phenylcoumarins as potent and selective MAO-B inhibitors', *Bioorg. Med. Chem. Lett.* **2009**, 19, 5053–5055.
- [16] M. J. Matos, D. Viña, P. Janeiro, F. Borges, L. Santana, E. Uriarte, 'New halogenated 3-phenylcoumarins as potent and selective MAO-B inhibitors', *Bioorg. Med. Chem. Lett.* **2010**, 20, 5157–5160.
- [17] S. Serra, G. Ferino, M. João Matos, S. Vázquez-Rodríguez, G. Delogu, D. Viña, E. Cadoni, L. Santana, E. Uriarte, 'Hydroxycoumarins as selective MAO-B inhibitors', *Bioorg. Med. Chem. Lett.* **2012**, 22, 258–261.
- [18] L. Piazza, A. Cavalli, F. Colizzi, F. Belluti, M. Bartolini, F. Mancini, M. Recanatini, V. Andrisano, A. Rampa, 'Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds', *Bioorg. Med. Chem. Lett.* **2008**, 18, 423–426.
- [19] C. A. Kontogiorgis, Y. Xu, D. Hadjipavlou-Litina, Y. Luo, 'Coumarin derivatives protection against ROS production in cellular models of A β toxicities', *Free Radical Res.* **2007**, 41, 1168–1180.
- [20] A. Ayati, T. O. Bakhshaiesh, S. Moghimi, R. Esmaeili, K. Majidzadeh-A, M. Safavi, L. Firoozpour, S. Emami, A. Foroumadi, 'Synthesis and biological evaluation of new coumarins bearing 2,4-diaminothiazole-5-carbonyl moiety', *Eur. J. Med. Chem.* **2018**, 155, 483–491.
- [21] L. Jalili-Baleh, H. Forootanfar, T. T. Küçükılınç, H. Nadri, Z. Abdolahi, A. Ameri, M. Jafari, B. Ayazgok, M. Baeeri, M. Rahimifard, S. N. A. Bukhari, M. Abdollahi, M. R. Ganjali, S. Emami, M. Khoobi, A. Foroumadi, 'Design, synthesis and evaluation of novel multi-target-directed ligands for treatment of Alzheimer's disease based on coumarin and lipoic acid scaffolds', *Eur. J. Med. Chem.* **2018**, 152, 600–614.
- [22] F. Mehrabi, Y. Pourshojaei, A. Moradi, M. Sharifzadeh, L. Khosravani, R. Sabourian, S. Rahmani-Nezhad, M. Mohammadi-Khanaposhtani, M. Mahdavi, A. Asadipour, H. R. Rahimi, S. Moghimi, A. Foroumadi, 'Design, synthesis, molecular modeling and anticholinesterase activity of benzylidene-benzofuran-3-ones containing cyclic amine side chain', *Future Med. Chem.* **2017**, 9, 659–671.
- [23] M. Pohanka, 'Alzheimer's disease and oxidative stress: a review', *Curr. Med. Chem.* **2014**, 21, 356–364.
- [24] L. M. Kabeya, A. A. de Marchi, A. Kanashiro, N. P. Lopes, C. H. T. P. da Silva, M. T. Pupo, Y. M. Lucisano-Valim, 'Inhibition of horseradish peroxidase catalytic activity by new 3-phenylcoumarin derivatives: Synthesis and structure-activity relationships', *Bioorg. Med. Chem.* **2007**, 15, 1516–1524.
- [25] W. Luo, Y.-B. Su, C. Hong, R.-G. Tian, L.-P. Su, Y.-Q. Wang, Y. Li, J.-J. Yue, C.-J. Wang, 'Design, synthesis and evaluation of novel 4-dimethylamine flavonoid derivatives as potential multi-functional anti-Alzheimer agents', *Bioorg. Med. Chem.* **2013**, 21, 7275–7282.
- [26] G. L. Ellman, K. D. Courtney, V. Andres Jr., R. M. Featherstone, 'A new and rapid colorimetric determination of acetylcholinesterase activity', *Biochem. Pharmacol.* **1961**, 7, 88–95.
- [27] Z. Datki, A. Juhász, M. Gálfi, K. Soós, R. Papp, D. Zádori, B. Penke, 'Method for measuring neurotoxicity of aggregating

- polypeptides with the MTT assay on differentiated neuroblastoma cells', *Brain Res. Bull.* **2003**, *62*, 223–229.
- [28] H. LeVine, 'Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution', *Protein Sci.* **1993**, *20*, 404–410.
- [29] O. Trott, A. J. Olson, 'AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading', *J. Comput. Chem.* **2010**, *31*, 455–461.
- [30] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, 'AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility', *J. Comput. Chem.* **2009**, *30*, 2785–2791.

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