Acetylcholinesterase Inhibition, Molecular Docking and ADME Prediction Studies of New Dihydrofuran-Piperazine Hybrid Compounds

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**Acetylcholinesterase inhibition, molecular docking and ADME prediction studies of
new dihydrofuran-piperazine hybrid compounds**

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Abstract

Novel acrylamide and methacryloyl carrying piperazine-dihydrofuran derivatives (**3a-p**) were designed and obtained from radical cyclizations of unsaturated piperazine derivatives (**1a-f**) with 1,3-dicarbonyl compounds (**2a-c**) mediated by $\text{Mn}(\text{OAc})_3$. Structures of obtained compounds were confirmed with ^1H NMR (proton nuclear magnetic resonance), ^{13}C NMR (Carbon-13 nuclear magnetic resonance), HRMS (High resolution mass spectrometry), FTIR (Fourier-transform infrared spectroscopy and melting point analysis. Inhibitory activities of all piperazine-dihydrofuran compounds were evaluated against AChE (Acetylcholinesterase) by Ellman method and test results showed that **3a**, **3c**, **3j** and **3l** are most active AChEI's (AChE inhibitors) of our work with IC_{50} (half-maximal inhibitory concentration) values of 2.62, 5.29, 1.17 and 3.90 μM , respectively. Furthermore, ligand-protein interactions and inhibitory activity mechanisms of **3a** and **3j** were investigated by molecular docking. Finally, in silico molecular property and ADME (absorption, distribution, metabolism and excretion) of potential AChEI's (AChE inhibitor) were predicted by PreADMET and Molinspiration web servers. It can be concluded that the lead compound **3j** shows excellent inhibition and satisfactory druglike characteristics.

Keywords

Piperazine, Dihydrofuran, Radical cyclization, Acetylcholinesterase inhibition, Molecular docking, ADME

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and is one of the main cause of dementia effecting elderly people. The illness is characterized with executive disorders, memory loss, mood disturbances, depression and progressive loss of cognitive abilities [1,2]. Based on the report of World Health Organization (WHO) about AD, about 36 million people around globe were suffering from dementia until 2010 and this would be increased to 66 million by 2030 [3]. Many theories were suggested to clarify the exact origin of AD such as cholinergic transmission [4], tau protein hyperphosphorylation [5] and beta-amyloid aggregation [6]. Among them cholinergic transmission is the most commonly accepted theory and increasing levels of neurotransmitter acetylcholine in brain is crucial for the treatment of AD [7-9].

Acetylcholinesterase (AChE) is an enzyme in cholinesterase family which catalyzes the rapid hydrolysis of neurotransmitter acetylcholine and terminates impulse transmission at cholinergic synapses [10]. Inhibiting AChE is the most prominent way in the field of AD treatment [11] and there are many commercially available inhibitor drugs such donepezil [12], rivastigmine [13] and galantamine [14].

Heterocycles bearing nitrogen are important compounds in the field of medicinal chemistry and widely used for their biological properties [15]. Piperazine is considered a privileged structure for its ability of binding to multiple structures with high affinity [16]. There are many biological activities reported for compounds bearing piperazine moieties such as anticonvulsant [17], antibacterial [18], antituberculosis [19], antiviral [20], anticancer [21] and antimalarial [22]. Also many AChE inhibition studies were performed for piperazine derivatives [23-25].

Dihydrofurans are biologically active heterocycles and useful building blocks for naturally occurring compounds such as Sarcophytoxide [26], Clerodin [27], Fercoprolone [28] and Austocystin [29]. Dihydrofurans can be obtained by C-C bond forming radical cyclization reactions that occur through the addition of α -carbon radicals to unsaturated systems [30-32].

Alpha carbon radicals are generated from active methylene compounds via single electron transferring transition metal salts (Mn^{3+} , Ce^{4+} , Co^{3+} , etc). Manganese(III) acetate [33-37] and Cerium(IV) ammonium nitrate [38-40] are the most widely used transition metal salts in this reaction. Our research group has reported the synthesis of dihydrofuran compounds by the radical cyclization of 1,3-dicarbonyl compounds with various unsaturated systems such as aromatic conjugated alkenes [41,42], dienes [43,44] and conjugated amide derivatives [45,46] mediated by Manganese(III) acetate and Cerium(IV) ammonium nitrate.

Over recent years there are some works in literature about AChE inhibition of acrylamide and acrylamide containing piperazine compounds. Pan and coworkers described the synthesis of ferulic acid-memoquin hybrids which contains aromatic acrylamide moieties and evaluated their inhibition capabilities against AChE and reported IC_{50} values between 3.2-34.7 μM [47]. Additionally, Shaik and coworkers designed flavone-8-acrylamide compounds and obtained inhibition results between 0.064-2.81 μM [48]. Moreover, cinnamic N-benzylpiperidine hybrids were synthesized by Estrada et al. and they obtained good inhibition results (IC_{50} = 0.26-8.73) [49]. Finally aromatic acrylamide carrying piperazine derivatives were obtained by Singh and coworkers that show AChE inhibition with IC_{50} values between 9.91-29.34 μM [50].

Our research group reported aromatic amide substituted piperazine-dihydrofuran derivatives [51] and good inhibition results were reported (IC_{50} values between 2.24-17.93 μM). Encouraged by the results of our previous work and based on the results that were acquired by many research groups for acrylamides and piperazine compounds over recent years, in present work we studied the radicalic cyclization reaction between aromatic substituted acrylamide and methacryloyl carrying piperazine compounds (**1a-f**) with enolizable 1,3-dicarbonyls (dimedone (**2a**), acetylacetone (**2b**), ethylacetoacetate (**2c**)) *via* $\text{Mn}(\text{OAc})_3$. Radicalic cyclization is possible to occur on both methacryloyl and substituted acrylamide sides depending on steric hindrance and radical stability. Mechanism of this reaction is discussed in Results and

Discussion section. General molecular structures of obtained piperazine-dihydrofuran molecules can be seen in Scheme 1. Acrylamide and substituted acrylamide carrying piperazine-dihydrofuran products were obtained from these reactions and both of these final products (**3a-p**) were isolated and evaluated for AChE inhibitions.

Scheme 1 Molecular structures of substituted acrylamide (**A**) and methacryoyl (**B**) carrying piperazine-dihydrofuran compounds

To further understand the interaction of newly synthesized compounds with AChE, molecular docking studies were performed to investigate the binding modes with AChE (PDB: 4EY7) active site.

Moreover, to predict the druglike potentials of obtained compounds, in silico molecular property and ADME (absorption, distribution, metabolism, excretion) prediction studies were conducted to predict druglikeness of obtained compounds by using Molinspiration and PreADMET online servers.

Results and Discussion

Chemistry

The reactions of acrylamide substituted piperazines (**1a-e**) with dimedone (**2a**) and acetylacetone (**2b**) were given at Table 1. The reaction of piperazine derivative **1a** with dimedone (**2a**) gave piperazine substituted dihydrofuran compounds **3a** (10%) and **3b** (45%) from the cyclization of each acyl group and these compounds were differentiated by their ¹H NMR spectra. The ¹H NMR spectrum of **3a** shows trans alkene protons at 6.85 and 7.72 ppm as doublet ($J = 15.6$ Hz) for each proton. Also, geminal protons of dihydrofuran ring can be seen at 2.72 and 3.50 ppm as doublet ($J = 15.2$ Hz) for each proton. The terminal alkene protons of **3b** is resonated at 5.03 and 5.20 ppm as two singlet. Also, vicinal protons of dihydrofuran moiety of **3b** can be seen at 4.23 and 6.11 ppm as two doublet ($J = 5.6$ Hz).

Reactions of piperazine derivatives **1b** and **1c** with dimedone (**2a**) gave acrylamide piperazine substituted dihydrofurans **3c** (45%) and **3d** (60%), respectively. Both radical cyclizations occurred through the methacryloyl group, regioselectively. However, the reaction of methacryloyl and (2*E*),(4*E*)-5-phenyl-2,4-pentadienoyl substituted compound **1d** with **2a** formed compound **3e** (20%) through 2,4-pentadienoyl. The exact structure of this compound was clarified with ¹H NMR and HMBC spectra. Also, the reaction of **1e** with **2a** gave piperazine substituted dihydrofurans **3f** (10%) and **3g** (40%) from the cyclizations of each acyl group on **1e**. In addition, **3h** (45%) and **3i** (30%) were obtained from the reaction of acetylacetone (**2b**) with **1a** and **1c**, respectively.

Table 1 Synthesis of piperazine-dihydrofurans (**3a-i**).

Entry	Piperazines	1,3-dicarbonyls	Products and yields ^a	
1	1a	2a	3a, 10%	3b, 45%
2	1b	2a	3c, 45%	
3	1c	2a	3d, 60%	
4	1d	2a	3e, 20%	
5	1e	2a	3f, 10%	3g, 40%
6	1a	2b	3h, 45%	
7	1c	2b	3i, 30%	

a) Isolated yields based on 1,3-dicarbonyl compounds

As can be seen at Table 2, while piperazine-dihydrofurans **3j** (13%) and **3k** (25%) were obtained from the cyclization (through both acyl groups) of **1a** with **2c**, **3l** (40%) and **3m** (50%) were isolated from the cyclization (only through methacryloyl group) of **1b** and **1c** with **2c**. Similarly, while the reaction of **1f** with **2c** gave piperazine dihydrofurans **3n** (20%) and **3o** (30%), only **3p** (20%) was isolated from the reaction of **1e** with **2c**.

Table 2 Synthesis of piperazine-dihydrofurans (**3j-p**).

Entry	Piperazines	1,3-dicarbonyls	Products and yields	
1	1a	2c	3j, 13%	3k, 25%
2	1b	2c	3l, 40%	
3	1c	2c	3m, 50%	
4	1f	2c	3n, 20%	3o, 30%
5	1e	2c	3p, 20%	

a) Isolated yields based on 1,3-dicarbonyl compounds.

Scheme 2 Proposed mechanism of $\text{Mn}(\text{OAc})_3$ mediated radical cyclization.

The proposed mechanism for the formation of dihydrofurans is explained in Scheme 2 [52]. According to this mechanism, the enol form of dimedone (**A**) reacts with $\text{Mn}(\text{OAc})_3$ and an alpha carbon radical **B** is formed, while Mn^{3+} reduces to Mn^{2+} . Alpha carbon radical can interact with both unsaturated sides of piperazine compound and both of these pathways (*i* and *ii*) are likely to occur at the same time. On pathway *i* an electron from alkene is added to this α -carbon

radical and produces the radical carbon intermediate **C**. Intermediate **C** oxidizes to carbocation **D** with $\text{Mn}(\text{OAc})_3$ and intramolecular cyclization of **D** forms the product **E**. On pathway *ii* radicalic cyclization reaction follows similar steps on the other unsaturated site and product **H** is formed.

In vitro inhibition results of piperazine-dihydrofuran compounds against AChE

Starting acylated piperazine derivatives (**1a-f**) used in this study were tested against AChE and they show almost no inhibition ($\text{IC}_{50} > 100 \mu\text{M}$). On the other hand, in vitro inhibition capabilities of some of the obtained piperazine substituted dihydrofurans were proved to be significantly high. All results compared to standard drugs Donepezil and Galanthamine were presented in Table 3.

Table 3 IC_{50} values of piperazine-dihydrofuran compounds (**3a-p**) towards AChE.

Compound	$\text{IC}_{50} \pm \text{SD} (\mu\text{M})^a$
3a	2.62 ± 0.2
3b	>100
3c	5.29 ± 0.5
3d	11.89 ± 1.3
3e	>100
3f	8.55 ± 0.6
3g	>100
3h	>100
3i	>100
3j	1.17 ± 0.7
3k	>100
3l	3.90 ± 0.5
3m	8.36 ± 0.4
3n	6.11 ± 0.6
3o	>100
3p	8.42 ± 0.3
Donepezil[53]	0.041
Galanthamine[53]	1.65

^aThe values are mean of three independent experiments $\pm$ SD

IC_{50} values of cinnamoyl acrylamide substituted **3a** and methacryloyl substituted **3b** were calculated and while **3a** has IC_{50} value of $2.62 \mu\text{M}$, **3b** has almost no inhibition ($\text{IC}_{50} > 100 \mu\text{M}$). Also, IC_{50} values for compounds **3c** and **3d** are 5.19 and $11.89 \mu\text{M}$, respectively. By comparing these IC_{50} values with **3a** ($\text{IC}_{50} = 2.62 \mu\text{M}$) it can be seen that inhibition powers of

3c and **3d** are lower than **3a**. In addition, while IC_{50} value of **3f** was calculated as 8.55 μ M, it is $> 100 \mu$ M for methacryloyl containing compound **3g**. Similarly, compound **3e** shows almost no inhibition ($IC_{50} > 100 \mu$ M). Also, contrary to products obtained from dimedone, **3h** and **3i**, which obtained from the reactions of acetylacetone (**2b**), show no inhibition effects.

Among the piperazine-dihydrofuran compounds obtained from the reactions of ethyl acetoacetate (**2c**) with acrylamide piperazines (**1a-e**), compounds **3j** ($IC_{50} = 1.17 \mu$ M) and **3l** ($IC_{50} = 3.90 \mu$ M) show best inhibition effects. Also, IC_{50} values of **3m**, **3n** and **3p** were calculated as 8.36, 6.11 and 8.42 μ M, respectively. On the other hand, similar to compounds mentioned above, methacryloyl containing compounds **3k** and **3o** has almost no inhibition effect ($IC_{50} > 100\mu$ M). In the light of these informations, it is clear that, aromatic moiety carrying acrylamide substituents on piperazine-dihydrofuran compounds have significantly positive effect on inhibitions. Also, it is concluded that, carboxylate substitution on dihydrofuran group increases inhibition efficiency than other substitutions on dihydrofurans. Based on these results, compound **3j** is selected as our lead compound.

Molecular docking results of selected piperazine-dihydrofuran compounds (3a and 3j)

Molecular docking studies were performed on two of our most potent inhibitor compounds (**3a** and **3j**). Docking procedure was validated by re-docking the native ligand donepezil to target AChE. Near perfect alignment with a RMSD value of 0.340 was obtained from validation results. Binding score of native ligand donepezil is -12.2 Kcal/mol. Binding energies for top docking poses of ligands **3a** and **3j** are -9.6 and -10.4 Kcal/mol, respectively. Docking poses of **3a**, **3j** and Donepezil are superpositioned in AChE active site cavity and shows good alignment with native ligand Donepezil.

Fig. 1 AChE active site cavity with donepezil (green), **3a** (magenta) and **3j** (cyan) inside.

Ligand-protein interactions of top binding poses of ligands **3a** and **3j** were given at Figure 2.

By investigating the docking mod of **3a** it can be seen that one of the methyls of dimedone ring of **3a** forms π -alkyl interactions with aromatic moiety of HIS287. Also, dimedone carbonyl forms a carbon-hydrogen bond with TRP286. Methyl group on dihydrofuran ring interacts with TYR341 through a hydrophobic π -alkyl interaction. Piperazine ring forms hydrophobic π -alkyl interactions with aromatic moieties of TRP286 and carbon-hydrogen bonds with SER293 and TYR341. Additionally acrylamide carbonyl forms hydrogen bondings with PHE295 and ARG296. Finally aromatic moiety of acrylamide group forms π - π interactions with TYR341 and PHE338.

By investigating the interactions of **3j**, it can be seen that ethyl carboxylate moiety of **3j** forms π - σ interaction with aromatic moiety of TRP86. Also ester carbonyl forms a Carbon-Hydrogen bond with HIS447. Methyl groups on dihydrofuran ring made π -alkyl interactions with

PHE338, TYR124 and PHE297, also one methyl group on dihydrofuran ring interacts with TYR341 through a π - σ interaction. Moreover carbonyl next to dihydrofuran ring forms a hydrogen bond with TYR124. Additionally, piperazine ring interacts with TYR341 and TRP286 through π -alkyl interactions. Finally, aromatic ring of cinnamoyl group interacts with LEU289 through a π -alkyl interaction.

By considering the ligand-protein interactions of top binding modes of ligands it can be seen that docking results support in vitro inhibition results.

Fig. 2 Ligand-protein interactions of **3a** (A) and **3j** (B).

In silico molecular property and ADME prediction results

ADME properties are one of the main reasons for a drug candidate to fail in clinical trials. In silico molecular property and ADME predictions of obtained piperazine-dihydrofuran compounds which have inhibition powers against AChE (AChEI's) (**3a**, **3c**, **3d**, **3f**, **3j**, **3l**, **3m**,

3m and **3p**) were carried out using Molinspiration (<https://www.molinspiration.com/>) and PreADMET (<https://preadmet.bmdrc.kr/>) webserver in order to predict druglikeness of these molecules. According to Lipinski's rule [54] a drug candidate has no more than one violation of the following criteria: (i) Hydrogen bond acceptors must be ≤ 10 . (ii) Hydrogen bond donors must be ≤ 5 (iii) Molecular weight (MW) must be less than 500 D and (iv) Octanol-water partition coefficient (MilogP) of the molecule must be ≤ 5 .

As can be seen in Table 4 Piperazine-dihydrofuran compounds show no violation against Lipinski's rule.

Table 4 In silico molecular property predictions for AChEI's.

Code	MW ^a	MiLogP ^b	HBA ^c	HBD ^d	Nviol ^e	TPSA ^f	MVol ^g
Rule	<500	≤ 5	≤ 10	≤ 5	≤ 1	<160 Å ²	
3a	422.52	3.26	6	0	0	66.92	398.16
3c	436.55	3.14	6	0	0	66.92	414.72
3d	498.62	4.36	6	0	0	66.92	469.57
3f	442.58	3.04	6	0	0	66.92	405.43
3j	412.49	3.15	7	0	0	76.16	384.68
3l	426.51	3.03	7	0	0	76.16	401.24
3m	488.58	4.25	7	0	0	76.16	456.09
3n	418.51	2.87	7	0	0	76.16	375.39
3p	432.54	2.93	7	0	0	76.16	391.95

a: Molecular weight (MW); b: logarithm of octanol-water partition coefficient (MiLogP); c: Number of hydrogen bond acceptors (HBA); d: Number of hydrogen bond donors (HBD); e: Lipinski rule violations (nviol); f: Topological polar surface area (TPSA); g: Molecular volume (MVol).

Moreover, in silico ADME prediction results of AChEI's were given at Table 5. [55]

Table 5 In silico ADME predictions of AChEI's.

Code	Absorption				Distribution
	Human intestinal absorption (%)	In vitro Caco-2 cell permeability (nm/s)	In vitro MDCK cell permeability (nm/s)	In vitro skin permeability (log Kp, cm/h)	In vitro plasma protein binding PPB (%)
Rule	0-20 (poor) 20-70 (moderate) 70-100 (well)	<4 (low), 4-70 (moderate), >70 (high)	<25 (low) 25-500 (moderate) >500 (high)		>90 (strongly bound) <90 (weakly bound)
3a	98.00	49.67	0.06	-3.29	82.79
3c	97.86	50.32	0.06	-3.25	84.87
3d	97.48	52.53	0.04	-2.39	90.62
3f	99.52	53.17	0.07	-3.85	84.09
3j	98.95	45.52	0.11	-3.17	76.39
3l	98.87	46.87	0.08	-3.14	79.85
3m	97.69	50.02	0.04	-2.36	89.16
3n	98.65	50.25	0.17	-3.75	72.94
3p	98.96	51.48	0.11	-3.71	78.50

Human intestinal absorption (HIA) indicates gastrointestinal permeation across membranes for drugs which taken orally. All AChEI compounds show great HIA values over 97%.

In vitro Caco-2 cell permeability is an indication of intestinal absorption of drugs. According to our results all AChEI's show moderate permeations between 41-53 nm/s.

In vitro MDCK cell permeability test utilizes canine kidney cells to test permeability. All AChEI test compounds show low permeation values.

Skin permeability is a factor that indicates delivery of a drug through transdermal administration. All AChEI's compounds show negative permeability which shows transdermal administration is not suitable for these molecules.

In vitro plasma protein binding (PPB) indicates percentage of a drug is bound to blood plasma proteins. Our AChEI's show binding values less than 90% which means they can efficiently diffuse to cell membranes.

Conclusion

In the presented work, new piperazine-dihydrofuran compounds (**3a-p**) were designed and synthesized from $\text{Mn}(\text{OAc})_3$ mediated radical cyclizations of 1,3-dicarbonyl compounds (**2a-c**) and acrylamide carrying piperazine derivatives (**1a-f**) in low and medium yields. AChE inhibition capabilities of starting piperazine derivatives (**1a-f**) and piperazine-dihydrofuran compounds (**3a-p**) were tested. Although many of the piperazine-dihydrofuran compounds (**3a**, **3c**, **3d**, **3f**, **3j**, **3l**, **3m**, **3n** and **3p**) show inhibition capabilities against AChE, starting acylated piperazine compounds (**1a-f**) show no inhibition effects. While piperazine-dihydrofuran compounds containing aromatically substituted acrylamide moieties has high inhibition effects against AChE (IC_{50} values ranging from 1.17 to 11.89 μM) methacryloyl carrying piperazine-dihydrofuran compounds (**3b**, **3g**, **3h**, **3i**, **3k** and **3o**) show almost no inhibitions. Also, carbethoxy substituted piperazine-dihydrofuran compounds show higher inhibition effects than other piperazine-dihydrofurans, especially **3j** ($\text{IC}_{50} = 1.17 \mu\text{M}$) which is our lead compound. In addition, molecular docking studies were performed with the lead compound **3j** and the other most potent AChEI **3a** to investigate ligand-protein interactions and binding energies. Calculated docking results were compared to standard drug Donepezil. Binding scores of Donepezil is -12.2 Kcal/mol and -9.6, -10.4 Kcal/mol for **3a** and **3j** respectively. Finally, in silico molecular property analysis and ADME prediction studies show that our lead compound **3j** and other AChEI's have satisfactory druglike characteristics. Summarily, the lead piperazine-dihydrofuran compound **3j** which carries phenyl substituted acrylamide moiety and carboxylate group have excellent AChE inhibition and satisfactory druglike characteristics. This compound have potential to be a drug candidate and can be further modified to increase the activity against Acetylcholinesterase.

Experimental

All reagents and solvents are commercially available and analytically pure unless otherwise stated. AChE (from electric eel, type V-S), acetylthiocholine iodide (ATCI), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were supplied from Sigma Aldrich. Radical oxidant $\text{Mn}(\text{OAc})_3$ was synthesized by electrochemical method [43].

^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-400 High performance Digital FT-NMR and Varian Oxford NMR300 spectrometers. HRMS spectra were obtained on an Agilent 1200/6210 LC/MS spectrophotometer. IR spectra (ATR) were obtained with a Bruker Tensor27 spectrophotometer in the $400\text{--}4000\text{ cm}^{-1}$ range with 2 cm^{-1} resolutions. UV absorbance was recorded by Rigol Ultra-3000 UV-Vis Spectrophotometer. Melting points were determined on a Gallenkamp capillary melting point apparatus. Thin layer chromatography (TLC) was performed on Merck aluminum-packed silica gel plates. Purification of products were performed by column chromatography on silica gel (Merck silica gel 60, $40\text{--}60\text{ }\mu\text{m}$) or preparative TLC on silica gel of Merck (PF254-366 nm).

General synthesis procedure and spectroscopic data of piperazine dihydrofuran compounds (3a-p)

Starting unsaturated piperazine derivatives (**1a-f**) were obtained according to our previously reported work [56]. All piperazine-dihydrofuran compounds (**3a-p**) were synthesized by the general method described below.

$[\text{Mn}(\text{OAc})_3] \cdot 2\text{H}_2\text{O}$ (2 mmol, 0.53 g) in 15 mL glacial acetic was heated to $80\text{ }^\circ\text{C}$ until dissolved. After that the solution temperature was cooled to $65\text{ }^\circ\text{C}$ and a solution of 1,3-dicarbonyl compound (**2a-c**) (1mmol) and piperazine compound (**1a-f**) (1.2 mmol) in 3 mL of acetic acid was added. The mixture was stirred and disappearance of the initial dark brown indicated that the reaction was finished (10-30 min). After that, water was added and the reaction mixture was extracted with CHCl_3 ($3 \times 20\text{ mL}$). The combined organic phase was neutralised with saturated

NaHCO₃ solution, dried over anhydrous Na₂SO₄ and evaporated. The crude product was purified with column chromatography or preparative TLC (chloroform:acetone (85:15) as eluent).

2-(4-cinnamoylpiperazine-1-carbonyl)-2,6,6-trimethyl-3,5,6,7-tetrahydrobenzofuran-4(2H)-one (3a)

It was obtained as a yellow oil; yield: 10% (42 mg); IR (ATR) $\nu_{\max}$ 3071, 2961, 2921, 2850, 1725 (C=O), 1652 (C=O), 1630 (C=C), 1226, 1192, 752, 692 (aromatic C-H) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.72 (1H, d, J = 15.6 Hz, H_{olef.}), 7.53 (2H, dd, J = 5.2, 2 Hz, arom. CH), 7.37 (3H, dd, J = 5.2, 2 Hz), 6.85 (1H, d, J = 15.6 Hz, H_{olef.}), 3.87-3.58 (8H, broad), 3.50 (1H, d, J = 15.2 Hz, Ha-3), 2.72 (1H, d, J = 15.2 Hz, Hb-3), 2.30 (2H, d, J = 16.0 Hz), 2.27 (2H, s), 1.63 (3H, s, -CH₃), 1.13 (3H, s, -CH₃), 1.11 (3H, s, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 194.6 (C=O), 173.0 (C=C, C-7a), 169.8 (C=O), 165.7 (C=O), 143.7 (C=C), 134.9, 129.9, 128.9, 127.8, 116.3 (C=C), 111.4 (C=C, C-3a), 91.9, 50.9, 46.0, 37.8, 37.6, 34.3, 28.7 (-CH₃), 28.6 (-CH₃), 26.2 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₅H₃₀N₂O₄ 423.22783 found: 423.22835 (M + H)⁺

Trans-3-(4-methacryloylpiperazine-1-carbonyl)-6,6-dimethyl-2-phenyl-3,5,6,7-tetrahydrobenzofuran-4(2H)-one (3b)

It was obtained as a yellow oil; yield: 45% (190 mg); IR (ATR) $\nu_{\max}$ 3054, 2961, 2952, 2868, 1719 (C=O), 1637 (C=O), 1610 (C=C), 1228, 1208, 760, 706 (arom. CH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.41-7.20 (3H, m, arom. CH), 7.25-7.23 (2H, m, arom. CH), 6.11 (1H, d, J = 5.6 Hz, H-2), 5.20 (1H, s, H_{olef.}), 5.03 (1H, s, H_{olef.}), 4.23 (1H, d, J = 5.6 Hz, H-3), 4.01-3.30 (8H, broad), 2.47 (2H, d, J = 16.0 Hz), 2.26 (2H, d, J = 16.0 Hz), 1.94 (3H, s, -CH₃), 1.15 (3H, s, -CH₃), 1.04 (3H, s, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 193.9 (C=O), 178.1 (C=C, C-7a), 171.3 (C=O), 170.3 (C=O), 140.0 (C=C), 139.7, 129.1, 128.9, 125.52, 116.0

(C=C), 112.1 (C=C, C-3a), 90.5, 51.1, 49.9, 47.2, 44.2, 37.9, 34.4, 28.9 (-CH₃), 28.3 (-CH₃), 20.4 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₅H₃₀N₂O₄ 423.22783 found: 423.22835 (M + H)⁺

2,6,6-trimethyl-2-(4-(3-phenylbut-2-enoyl)piperazine-1-carbonyl)-3,5,6,7-tetrahydrobenzofuran-4(2H)-one (3c)

It was obtained as a yellow oil; yield: 45% (196 mg); IR (ATR) $\nu_{\max}$ 3058, 2961, 2921, 2850, 1725 (C=O), 1652 (C=O), 1630 (C=C), 1225, 1192, 752, 692 (arom. CH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.45 (2H, d, *J* = 8.4, 2 Hz, arom. CH), 7.39-7.33 (3H, m, arom. CH), 6.26 (1H, s, H_{olef}), 3.80-3.54 (8H, broad), 3.49 (1H, d, *J* = 15.2 Hz, Ha-3), 2.71 (1H, d, *J* = 15.2 Hz, Hb-3), 2.29 (4H, s), 2.24 (3H, s, -CH₃), 1.62 (3H, s, -CH₃), 1.12 (3H, s, -CH₃), 1.10 (3H, s, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 194.6 (C=O), 173.0 (C=C, C-7a), 169.75 (C=O), 167.31 (C=O), 141.4 (C=C), 128.6, 128.5, 126.0, 118.8, 116.1 (C=C), 111.4 (C=C, C-3a), 91.9, 50.9, 46.0, 43.7, 37.8, 34.3, 28.7 (-CH₃), 28.6 (-CH₃), 26.3 (-CH₃), 18.06 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₆H₃₂N₂O₄ 437.24348 found 437.24483 (M + H)⁺

2-(4-(3,3-diphenylacryloyl)piperazine-1-carbonyl)-2,6,6-trimethyl-3,5,6,7-tetrahydrobenzofuran-4(2H)-one (3d)

It was obtained as a yellow oil; yield: 60% (300 mg); IR (ATR) $\nu_{\max}$ 3058, 2961, 2925, 2872, 1714 (C=O), 1632 (C=O), 1604 (C=C), 1201, 1006, 752, 701 (arom. CH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.37-7.26 (10H, m, arom. CH), 6.30 (1H, s, H_{olef}), 3.48-2.98 (8H, broad), 3.39 (1H, d, *J* = 15.2 Hz, Ha-3), 2.46 (1H, d, *J* = 15.2 Hz, Hb-3), 2.25 (2H, d, *J* = 16.4 Hz), 2.20 (2H, d, *J* = 16.4 Hz), 1.53 (3H, s, -CH₃), 1.11 (3H, s, -CH₃), 1.08 (3H, s, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 194.5 (C=O), 173.0 (C=C, C-7a), 169.4 (C=O), 167.34 (C=O), 147.9 (C=C), 140.5, 138.7, 129.5, 128.8, 128.4, 128.4, 128.0, 111.4 (C=C), 110.0 (C=C, C-3a), 91.7, 50.88, 46.1, 45.5, 42.9, 41.1, 37.7, 37.5, 34.2, 28.72 (-CH₃), 28.6 (-CH₃), 26.16 (-CH₃); HRMS (ESI) (m/z) Calcd for C₃₁H₃₄N₂O₄ 499.25913 found 499.26110 (M + H)⁺

Trans-3-(4-methacryloylpiperazine-1-carbonyl)-6,6-dimethyl-2-styryl-3,5,6,7-tetrahydrobenzofuran-4(2H)-one (3e)

It was obtained as a yellow oil; yield: 20% (89 mg); IR (ATR) $\nu_{\max}$ 3067, 2965, 2925, 2854, 1734 (C=O), 1646 (C=O), 1626 (C=C), 1191, 1090, 754, 695 (arom. CH) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 7.31 (5H, m, arom. CH), 6.66 (1H, d, $J = 16.0$ Hz, H_{olef}), 6.20 (1H, dd, $J = 16.0, 7.6$ Hz, H_{olef}), 5.74 (1H, dd, $J = 7.6, 6.4$ Hz, H-2), 5.22 (1H, s), 5.05 (1H, s), 3.71 (1H, d, $J = 6.4$ Hz, H-3), 3.98-3.26 (8H, broad), 2.41 (2H, d, $J = 17.0$ Hz) 2.20 (2H, d, $J = 16.4$ Hz), 1.96 (3H, s, $-\text{CH}_3$), 1.14 (3H, s, $-\text{CH}_3$) 1.12 (3H, s, $-\text{CH}_3$); ^{13}C -NMR (100 MHz, CDCl_3), δ (ppm): 193.9 (C=O), 177.7 (C=C, C-7a), 171.3 (C=O), 170.2 (C=O), 140.0 (C=C), 135.3, 134.1, 128.8 (C=C), 128.7, 128.6, 126.8, 125.4 (C=C), 115.9 (C=C), 112.1 (C=C, C-3a), 90.23, 51.06, 47.6, 46.4, 42.3, 37.9, 34.3, 28.7 ($-\text{CH}_3$), 28.4 ($-\text{CH}_3$), 20.4 ($-\text{CH}_3$); HRMS (ESI) (m/z) Calcd for $\text{C}_{27}\text{H}_{32}\text{N}_2\text{O}_4$ 449.24348 found 449.24527 ($\text{M}+\text{H}$) $^+$

2,6,6-trimethyl-2-(4-(3-(thiophen-2-yl)but-2-enoyl)piperazine-1-carbonyl)-3,5,6,7-tetrahydrobenzofuran-4(2H)-one (3f)

It was obtained as a yellow oil; yield: 10% (44 mg); IR (ATR) $\nu_{\max}$ 3071, 2965, 2921, 2863, 1716 (C=O), 1650 (C=O), 1621 (C=C), 1194, 1017, 759, 692 (arom. CH) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 7.28 (1H, d, $J = 0.8$ Hz, arom. CH), 7.21 (1H, dd, $J = 4.0, 0.8$ Hz, arom. CH), 7.03 (1H, dd, $J = 6.0, 4.0$ Hz, arom. CH), 6.38 (1H, s, H_{olef}), 3.79-3.55 (8H, broad), 3.50 (1H, d, $J = 15.2$ Hz, $\text{H}_a\text{-3}$), 2.72 (1H, d, $J = 15.2$ Hz, $\text{H}_b\text{-3}$), 2.31 (2H, d, $J = 16.0$ Hz), 2.29 (2H, $J = 16.0$ Hz), 2.25 (3H, s, $-\text{CH}_3$), 1.62 (3H, s, $-\text{CH}_3$), 1.12 (3H, s, $-\text{CH}_3$), 1.11 (3H, s, $-\text{CH}_3$); ^{13}C -NMR (100 MHz, CDCl_3), δ (ppm): 194.6 (C=O), 172.9 (C=C, C-7a), 171.9 (C=O), 169.7 (C=O), 145.1 (C=C), 127.8, 125.8, 125.6, 116.1 (C=C), 111.5 (C=C, C-3a), 91.9, 50.9, 46.3, 45.8, 37.8, 37.5, 34.2, 29.6 ($-\text{CH}_3$), 28.6 ($-\text{CH}_3$), 26.2 ($-\text{CH}_3$), 17.8 ($-\text{CH}_3$); HRMS (ESI) (m/z) Calcd for $\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_4\text{S}$ 443.19990 found 443.20162 ($\text{M} + \text{H}$) $^+$

3-(4-methacryloylpiperazine-1-carbonyl)-2,6,6-trimethyl-2-(thiophen-2-yl)-3,5,6,7-tetrahydrobenzofuran-4(2H)-one (3g)

It was obtained as a yellow oil; yield: 40% (177 mg); IR (ATR) $\nu_{\max}$ 3071, 2956, 2921, 2859, 1736 (C=O), 1643 (C=O), 1610 (C=C), 1194, 1024, 755, 700 (arom CH) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 7.28 (1H, d, $J = 4.4$ Hz, arom. CH), 7.00-6.97 (2H, m, arom. CH), 5.22 (1H, s, H_{olef}), 5.05 (1H, s, H_{olef}), 4.46 (1H, s, H-3), 3.60-3.52 (8H, broad), 2.40 (2H, d, $J = 16.4$ Hz), 2.27 (2H, d, $J = 16.4$ Hz), 1.95 (3H, s, $-\text{CH}_3$), 1.83 (3H, s, $-\text{CH}_3$), 1.46 (3H, s, $-\text{CH}_3$), 1.21 (3H, s, $-\text{CH}_3$); ^{13}C -NMR (100 MHz, CDCl_3) δ (ppm): 194.1 (C=O), 175.6 (C=C, C-7a), 171.3 (C=O), 167.94 (C=O), 148.9, 139.9 (C=C), 127.0, 125.4, 123.2, 116.1 (C=C), 112.7 (C=C, C-3a), 90.8, 53.0, 50.73, 46.28, 42.66, 37.7, 34.6, 28.7 ($-\text{CH}_3$), 28.4 ($-\text{CH}_3$), 23.8 ($-\text{CH}_3$), 20.45 ($-\text{CH}_3$); HRMS (ESI) (m/z) Calcd for $\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_4\text{S}$ 443.19990 found 443.20162 ($\text{M} + \text{H}$) $^+$

1-(4-(4-acetyl-2,5-dimethyl-2,3-dihydrofuran-2-carbonyl)piperazin-1-yl)-3-phenylprop-2-en-1-one (3h)

It was obtained as a yellow oil; yield: 45% (172 mg); IR (ATR) $\nu_{\max}$ 3076, 2965, 2912, 2845, 1714 (C=O), 1632 (C=O), 1602 (C=C), 1230, 1022, 756, 708 (arom. CH) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 7.72 (1H, d, $J = 15.2$ Hz, H_{olef}), 7.54-7.52 (2H, m, arom. CH), 7.41-7.36 (3H, m, arom. CH), 7.86 (1H, d, $J = 15.2$ Hz, H_{olef}), 3.78 (1H, d, $J = 14.8$ Hz, H-3), 3.98-3.56 (8H, broad), 2.79 (1H, d, $J = 14.8$ Hz, H-3), 2.23 (3H, s, $-\text{CH}_3$), 2.20 (3H, s, $-\text{CH}_3$), 1.62 (3H, s, $-\text{CH}_3$); ^{13}C -NMR (100 MHz, CDCl_3) δ (ppm): 191.0 (C=O), 177.5 (C=C, C-5), 169.7 (C=O), 165.5 (C=O), 143.7 (C=C), 134.9, 129.9, 128.8, 127.8, 116.3 (C=C), 114.2 (C=C, C-4), 88.6, 46.5, 44.3, 42.0, 29.6 ($-\text{CH}_3$), 26.1 ($-\text{CH}_3$), 24.5 ($-\text{CH}_3$), 14.84 ($-\text{CH}_3$); HRMS (ESI) (m/z) Calcd for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_4$ 383.19653 found 383.19745 ($\text{M} + \text{H}$) $^+$

1-(4-(4-acetyl-2,5-dimethyl-2,3-dihydrofuran-2-carbonyl)piperazin-1-yl)-3,3-diphenylprop-2-en-1-one (3i)

It was obtained as a yellow oil; yield: 30% (137 mg); IR (ATR) $\nu_{\max}$ 3058, 2978, 2925, 2863, 1725 (C=O), 1630 (C=O), 1604 (C=C), 1239, 1026, 759, 701 (arom. CH) cm^{-1} ; ^1H NMR (400

MHz, CDCl₃) δ (ppm): 7.33 (10H, m, arom CH), 6.30 (1H, s, H_{olef}), 3.69 (1H, d, J = 15.2 Hz, H-3), 3.79-2.78 (8H, broad), 2.71 (1H, d, J = 15.2 Hz, H_{olef}), 2.17 (6H, s, -CH₃) 1.63 (3H, s, -CH₃); ¹³C-NMR (100 MHz, CDCl₃), δ (ppm): 192.0 (C=O), 176.5 (C=C, C-5), 171.7 (C=O), 167.5 (C=O), 140.5, 138.7, 129.5, 128.8, 128.8, 128.4, 128.4, 128.1, 120.1, 117.5 (C=C), 114.8 (C=C, C-4), 88.5, 45.8, 41.9, 27.0, 25.3 (-CH₃), 23.4 (-CH₃), 12.2 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₈H₃₀N₂O₄ 459.22783 found 459.22902 (M+H)⁺

Ethyl 5-(4-cinnamoylpiperazine-1-carbonyl)-2,5-dimethyl-4,5-dihydrofuran-3-carboxylate (3j)

It was obtained as a yellow oil; yield: 13% (51 mg); IR (ATR) $\nu_{\max}$ 3067, 2969, 2930, 2872, 1736 (C=O), 1643 (C=O), 1608 (C=C), 1194, 1090, 761, 701 (arom. CH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.71 (1H, d, J = 15.2 Hz, H_{olef}), 7.53 (2H, dd, J = 5.2, 2 Hz arom. CH), 7.40-7.36 (3H, m, arom. CH), 6.87 (1H, d, J = 15.2 Hz, H_{olef}), 4.17 (2H, q, J = 7.2 Hz, -OCH₂CH₃), 3.92-3.55 (8H, broad), 3.61 (1H, d, J = 15.2 Hz, Ha-4) 2.74 (1H, d, J = 15.2 Hz, Hb-4), 2.21 (3H, s, -CH₃), 1.59 (3H, s, -CH₃) 1.27 (3H, t, J = 7.2 Hz, -OCH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃), δ (ppm): 170.5 (C=C, C-2), 165.7 (C=O), 165.5 (C=O), 164.5 (C=O), 143.5 (C=C), 134.9, 129.8, 129.0, 128.8, 127.8, 125.4, 116.4 (C=C), 102.4 (C=C, C-3), 88.3, 59.7, 46.2, 43.5, 42.2, 41.2, 26.0 (-CH₃), 14.3 (-CH₃), 14.1 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₃H₂₈N₂O₅ 413.20710 found 413.20625 (M+H)⁺

Trans-Ethyl 4-(4-methacryloylpiperazine-1-carbonyl)-2-methyl-5-phenyl-4,5-dihydrofuran-3-carboxylate (3k)

It was obtained as a yellow oil; yield: 25% (105 mg); IR (ATR) $\nu_{\max}$ 3026, 2966, 2930, 2870, 1740 (C=O), 1638 (C=O), 1610 (C=C), 1200, 1025, 750, 700 (arom CH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.42-7.33 (3H, m, arom. CH), 7.29-7.26 (2H, m, arom. CH), 5.67 (1H, d, J = 7.2 Hz, H-5), 5.21 (1H, s, H_{olef}), 5.02 (1H, s, H_{olef}), 4.35 (1H, d, J = 7.2 Hz, H-4), 4.15 (2H, q, J = 7.2 Hz, -OCH₂CH₃), 3.80-3.30 (8H, broad), 2.35 (3H, s, -CH₃), 1.94 (3H, s, -CH₃)

1.26 (3H, t, $J = 7.2$ Hz, $-\text{OCH}_2\text{CH}_3$); ^{13}C -NMR (100 MHz, CDCl_3), δ (ppm): 171.7 (C=C, C-2), 171.2 (C=O), 169.5 (C=O), 165.0 (C=O), 139.9 (C=C), 139.7, 129.0, 128.8, 127.8, 125.4, 116.0 (C=C), 103.65 (C=C, C-3), 87.3, 59.9, 46.1, 42.0, 20.4 ($-\text{CH}_3$), 14.56 ($-\text{CH}_3$), 14.43 ($-\text{CH}_3$); HRMS (ESI) (m/z) Calcd for $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_5$ 413.20710 found 413.20919 ($\text{M}+\text{H}$) $^+$

Ethyl-2,5-dimethyl-5-(4-(3-phenylbut-2-enoyl)piperazine-1-carbonyl)-4,5-dihydrofuran-3-carboxylate (3l)

It was obtained as a yellow oil; yield: 40% (170,8 mg); IR (ATR) ν_{max} 3054, 2961, 2916, 2868, 1732 (C=O), 1696 (C=O), 1617 (C=C), 1228, 1097, 750, 706 (arom. CH) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 7.45 (2H, dd, $J = 8.4, 1.6$ Hz, arom. CH), 7.36 (3H, m), 6.24 (1H, s, H_{olef}), 4.16 (2H, q, $J = 7.2$ Hz, $-\text{OCH}_2\text{CH}_3$), 3.92-3.51 (8H, broad), 3.61 (1H, d, $J = 15.2$ Hz, $\text{H}_{\text{a-4}}$), 2.73 (1H, d, $J = 15.2$ Hz, $\text{H}_{\text{b-4}}$), 2.29 (3H, s, $-\text{CH}_3$), 2.19 (3H, s, $-\text{CH}_3$), 1.58 (3H, s, $-\text{CH}_3$), 1.27 (3H, t, $J = 7.2$ Hz, $-\text{OCH}_2\text{CH}_3$); ^{13}C -NMR (100 MHz, CDCl_3), δ (ppm): 171.5 (C=C, C-2), 170.6 (C=O), 165.6 (C=O), 164.5 (C=O), 141.4 (C=C), 128.5, 128.5, 125.9, 118.8 (C=C), 102.3 (C=C, C-3), 88.3, 59.7, 46.3, 43.4, 41.2, 26.0 ($-\text{CH}_3$), 18.03 ($-\text{CH}_3$), 14.37 ($-\text{CH}_3$), 14.07 ($-\text{CH}_3$); HRMS (ESI) (m/z) Calcd for $\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_5$ 427.22275 found 427.22406 ($\text{M}+\text{H}$) $^+$

Ethyl 5-(4-(3,3-diphenylacryloyl)piperazine-1-carbonyl)-2,5-dimethyl-4,5-dihydrofuran-3-carboxylate (3m)

It was obtained as a yellow oil; yield: 50% (244 mg); IR (ATR) ν_{max} 3054, 2961, 2921, 2863, 1730 (C=O), 1650 (C=O), 1620 (C=C), 1228, 1060, 760, 703 (arom. CH) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 7.37-7.27 (10H, m, arom. CH), 6.30 (1H, s, H_{olef}), 4.15 (2H, q, $J = 6.8$ Hz, $-\text{OCH}_2\text{CH}_3$), 3.52 (1H, d, $J = 15.2$ Hz, H-4), 3.75-2.76 (8H, broad), 2.66 (1H, d, $J = 15.2$ Hz, H-4), 2.15 (3H, s, $-\text{CH}_3$), 1.50 (3H, s, $-\text{CH}_3$), 1.25 (3H, t, $J = 6.8$ Hz, $-\text{OCH}_2\text{CH}_3$); ^{13}C -NMR (100 MHz, CDCl_3), δ (ppm): 172.8 (C=C, C-2), 170.3 (C=O), 167.2 (C=O), 165.6 (C=O), 140.5, 129.5, 128.8, 128.7, 128.4, 128.4, 128.1, 120.0, 110.0 (C=C), 102.3 (C=C, C-3), 88.2, 59.7,

46.1, 45.6, 41.1, 25.9 (-CH₃), 14.3 (-CH₃), 14.0 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₉H₃₂N₂O₅ 489.23840 found 489.23854 (M+H)⁺

Ethyl-2,5-dimethyl-5-(4-(3-(thiophen-2-yl)acryloyl)piperazine-1-carbonyl)-4,5-dihydrofuran-3-carboxylate (3n)

It was obtained as a yellow oil; yield: 20% (84 mg); IR (ATR) $\nu_{\max}$ 3080, 2961, 2916, 2850, 2954, 1734 (C=O), 1694 (C=O), 1631 (C=C), 1236, 1090, 760, 701 (arom. CH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.84 (1H, d, J = 14.8 Hz, H_{olef}), 7.34 (1H, d, J = 4.4 Hz, arom. CH), 7.23 (1H, d, J = 3.2 Hz, arom. CH), 7.05 (1H, d, J = 3.2 Hz, arom. CH) 6.65 (1H, d, J = 14.8 Hz, H_{olef}), 4.17 (2H, q, J = 7.2 Hz, -OCH₂CH₃), 3.92-3.38 (8H, broad) 3.63 (1H, d, J = 15.2 Hz, Ha-4), 2.73 (1H, d, J = 15.2 Hz, Hb-4), 2.20 (3H, s, -CH₃), 1.59 (3H, s, -CH₃), 1.28 (3H, t, J = 7.2 Hz, -OCH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃) δ (ppm): 171.4 (C=C, C-2), 170.3 (C=O), 167.3 (C=O), 165.6 (C=O), 136.4, 130.6, 128.0, 127.5, 114.9 (C=C), 110.0 (C=C, C-3), 88.3, 59.7, 45.4, 42.3, 41.2, 29.68 (-CH₃), 14.37 (-CH₃), 14.09 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₁H₂₆N₂O₅S 419.16352 found 419.16496 (M+H)⁺

Trans-Ethyl 4-(4-methacryloylpiperazine-1-carbonyl)-2-methyl-5-(thiophen-2-yl)-4,5-dihydrofuran-3-carboxylate (3o)

It was obtained as a yellow oil; yield: 30% (125 mg); IR (ATR) $\nu_{\max}$ 3085, 2974, 2921, 2863, 1732 (C=O), 1692 (C=O), 1621 (C=C), 1194, 1080, 756, 703 (arom. CH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.33 (1H, dd, J = 4.8, 1.2 Hz, arom. CH), 7.17 (1H, dd, J = 4.8, 1.2 Hz, arom. CH), 7.00 (1H, dd, J = 4.8, 1.2 Hz, arom. CH), 5.90 (1H, d, J = 7.2 Hz, H-5), 5.22 (1H, s, H_{olef}), 5.03 (1H, s, H_{olef}), 4.50 (1H, d, J = 7.2 Hz, H-4), 4.14 (2H, q, J = 7.2 Hz, -OCH₂CH₃), 3.73-3.50 (8H, broad), 2.29 (3H, s, -CH₃), 1.94 (3H, s, -CH₃), 1.28 (3H, t, J = 7.2 Hz, -OCH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃) δ (ppm): 171.3 (C=C, C-2), 171.1 (C=O), 168.8 (C=O), 164.8 (C=O), 142.2, 139.9 (C=C), 127.1, 126.3, 126.05, 116.0 (C=C), 103.7 (C=C, C-

3), 83.0, 60.0, 53.1, 46.4, 42.7, 20.4 (-CH₃), 14.5 (-CH₃), 14.4 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₁H₂₆N₂O₅S 419.16352 found 419.16443 (M+H)⁺

Ethyl-2,5-dimethyl-5-(4-(3-(thiophen-2-yl)but-2-enoyl)piperazine-1-carbonyl)-4,5-dihydrofuran-3-carboxylate (3p)

It was obtained as a yellow oil; yield: 20% (86 mg); IR (ATR) $\nu_{\max}$ 3085, 2987, 2930, 2863, 1734 (C=O), 1694 (C=O), 1620 (C=C), 1194, 1062, 761, 706 (arom. CH) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.20 (1H, d, *J* = 0.8 Hz, arom. CH), 7.03-7.01 (2H, m, arom. CH), 6.37 (1H, s, H_{olef}), 4.16 (2H, q, *J* = 7.2 Hz, -OCH₂CH₃), 3.89-3.50 (8H, broad), 3.59 (1H, d, *J* = 15.2 Hz, Ha-4), 2.72 (1H, d, *J* = 15.2 Hz, Hb-4), 2.33 (3H, s, -CH₃), 2.26 (3H, s, -CH₃), 1.58 (3H, s, -CH₃), 1.27 (3H, t, *J* = 7.2 Hz, -OCH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃) δ (ppm): 170.5 (C=C, C-2), 166.6 (C=O), 165.6 (C=O), 164.5 (C=O), 145.1 (C=C), 140.6, 127.8, 126.9, 125.8, 116.25 (C=C), 102.3 (C=C, C-3), 88.3, 59.7, 46.3, 43.5, 41.2, 26.04 (-CH₃), 17.83 (-CH₃), 14.36 (-CH₃), 14.07 (-CH₃); HRMS (ESI) (m/z) Calcd for C₂₂H₂₈N₂O₅S 433.17917 found 433.18051 (M+H)⁺

Method of in vitro AChE inhibition experiments

Slightly modified Ellman method was carried out to determine in vitro AChE inhibitory activities of test compounds [57].

The assay solution was prepared by adding 1480 μ L of phosphate buffer (pH=8.0, 0.1 M), 50 μ L of DTNB solution (prepared with pH 7 phosphate buffer), 20 μ L of test compounds at desired concentration in ethanol-deionized water (1:1), 10 μ L of substrate solution (ATCI, in deionized water) and 25 μ L of AChE solution (prepared with deionized water and 1% gelatin). After that assay solution were incubated for 10 min. at 30 °C and absorbance at 412 nm were determined.

A control solution containing all compounds except inhibitor was performed same as above and the absorbance at 412 nm was considered 100% enzyme activity.

The percentage activity of AChE for any tested compound at desired concentration was calculated with the formula;

$$\% \text{ enzyme activity} = (A_s / A_0) \times 100$$

A_s : Absorbance of assay solution with inhibitor.

A_0 : Absorbance of control solution.

The concentration of each test compound was tested in triplicate and IC_{50} values were calculated graphically using GraphPad Prism 8.0.3 software. IC_{50} value is defined as the concentration of sample which performs 50% inhibition towards AChE.

Methods of in silico molecular docking experiments

Three dimensional structure of recombinant human AChE complexed with donepezil was obtained from the Protein Data Bank (4EY7) [58]. B-chain, water molecules and detergents were removed. Conformational analysis of inhibitor test compounds were performed with Avogadro software and most stable conformations were optimized with semiempirical PM6 method in Gaussian 09 Software. All ligand-protein docking calculations were performed as flexible ligand in rigid protein using AutoDock Vina software [59]. Best docking mod of ligand in terms of binding energy (Kcal/mol) was selected and used.

According to these results, it can be seen that all piperazine-dihydrofuran AChEI compounds presents satisfactory druglike properties, including our lead compound **3j**.

Supplementary Material

Copies of ^1H NMR, ^{13}C NMR and HRMS spectra for all novel compounds can be found as supplementary information.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1

AChE active site cavity with donepezil (green), 3a (magenta) and 3j (cyan) inside.

Figure 2

Ligand-protein interactions of 3a (A) and 3j (B).

Supplementary Files

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