Supporting Information to

Design, Synthesis and Biological Activity of Multifunctional α,β-Unsaturated Carbonyl Scaffolds for Alzheimer's Disease

by

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General Information - Synthesis – All building blocks and $CFCl_3$ used as ^{19}F NMR reference compound were purchased from Aldrich. $CDCl_3$ used as a solvent (99.8%) for the NMR studies was a Cambridge Isotope Laboratories product. Other solvents used in synthesis with minimum purity of 99.5% were from Fisher. The mass spectrometric identification of the products was carried out by an Agilent 6850 gas chromatograph – 5973 mass spectrometer system (70 eV electron impact ionization) using a 30m long DB-5 column (J&W Scientific). The ^1H, ^{13}C and ^{19}F NMR spectra were obtained on a 300 MHz superconducting Varian Gemini 300 NMR spectrometer, in DMSO(d_6) and $CDCl_3$ with tetramethylsilane and $CFCl_3$ as internal standards.

General Information - Assays – Galanthamine, used for comparison in AChE/BuChE assays and other components used in the assay were obtained from Sigma-Aldrich. AChE (6.0 IU/mg) derived from human erythrocytes was also purchased from Sigma Chemical. Butylcholinesterase (BuChE) obtained from equine serum was also purchased from Sigma. All chemicals, other than peptides, used in the assays were obtained from Sigma–Aldrich. Lyophilized synthetic Aβ(1-40) peptide (purity>95%) and N-α-Biotinyl-Aβ(1–42) (bio-Aβ42) were AnaSpec products. Fatty acid-free fraction V bovine serum albumin

was purchased from Boehringer–Mannheim. Streptavidin–HRP (SA–HRP) was a Rockland product. NeutrAvidin (NA) was obtained from Pierce. High-binding 9018 ELISA plates were purchased from Costar.

Synthesis

General procedure for the synthesis of compounds **1-15**

The solution of 3-methylacetophenone (1 mmole) in 10 ml CCl_4 was refluxed with N-bromosuccinamide (1 mmole) in presence of catalytic amount of benzoyl peroxide for 2-3 h. The reaction was monitored by TLC. The reaction mixture was filtered and concentrated under vacuum to yield 1-(3-(bromomethyl)phenyl)ethanone[1] as yellow oil, which was used for next reaction without further purification.

1-(3-(bromomethyl)phenyl)ethanone (1 mmole) was then refluxed with different substituted amines (1 mmole), respectively, in 20 ml CH_2Cl_2 to obtain the corresponding substituted amine in quantitative yield.[1] After the completion of reaction, the mixture was washed with water and extracted with CH_2Cl_2 to obtain the crude intermediate as a red slurry. This material was dissolved in anhydrous ether and cooled to 0 °C. To this solution ethereal HCl was added dropwise to precipitate the corresponding product as a hydrochloride salt, leaving behind the impurities in the solvent. The precipitate obtained was filtered and treated with 10% aq. NaOH. The free base thus obtained was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water followed by brine and dried over Na_2SO_4 and concentrated under vacuum to isolate the pure substituted amine. The resulting amine (1 mmole) was this dissolved in 25 ml EtOH. To this solution 10% aq. NaOH was added. The resulting reaction mixture was stirred at room temperature for 30 min. Then the reaction mixture was then cooled to 0-5 ºC. Corresponding aldehydes (1

mmol) dissolved in 10 ml ethanol was added dropwise to the mixture at 0-5 ºC. After completion of the addition, the reaction was stirred for 5-6 hours at room temperature. Then reaction mixture was concentrated to half of its initial volume, washed with water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was concentrated to obtain crude chalcones,[2] which were purified by column chromatography using ethyl acetate:hexane (3:7) as eluent (overall yields: 30-40%). All compounds were characterized by ^{1}H- NMR, ^{13}C-NMR spectroscopy. The spectral data of the compounds are listed at the end of the Supporting Information.

General procedure for the synthesis of compounds **16-22**

3-acetyl-7-methoxy-2H-chromen-2-one was obtained by the Knoevenagel condensation of 2-hydroxy-5-methoxybenzaldehyde with ethylacetoacetate. Ethereal solution of ethylacetoacetate (6.8 mmol) and piperidine (0.6 mmol) was stirred at 0°C. 2-hydroxy-5-methoxybenzaldehyde (1 mmol) was added dropwise at 0°C. The reaction mixture was stirred at room temperature for 2 h. At this stage solid precipitation was observed, which was filtered, washed with diethyl ether (2 x 5 ml) to isolate pure 3-acetyl-7-methoxy-2H-chromen-2-one as yellow solid (yield 91%).[3] The resulting compound (0.9 mmol) was then brominated using $Br_2/CHCl_3$ (0.46 mmol). The reaction mixture was stirred at room temperature overnight. At this stage solid precipitation was seen, which was filtered, washed with cold water, dried to obtain a brownish-orange solid (yield 82%).[4] 1 mmol of the brominated product thus obtained was reacted with 2 mmol of different amines in CH_2Cl_2 at room temperature for 3 h. Then the reaction mixture was washed with water and extracted with CH_2Cl_2 (3x10 ml). The combined CH_2Cl_2 extract was washed with

brine and dried over Na_2SO_4, concentrated under vacuum to obtain crude product, which was purified by column chromatography using ethyl acetate:hexane (1:1 followed by 6:4) eluent to isolate the product of desired purity (yield 60-70%). All compounds were characterized by ^{1}H- NMR, ^{13}C-NMR spectroscopy. The spectral data of the compounds are listed at the end of the Supporting Information.

Computational studies

Pharmacophore-based virtual screening of the designed molecules: Both the series of designed molecules were screened through the developed pharmacophore. The molecules were minimized using Ligprep and a maximum of 100 conformers were generated during the "Find matches" option with the distance matching tolerance of 1.0-1.5Å.

Molecular docking studies: Docking studies were carried out using standard Glide molecular docking protocol implemented within Maestro molecular modeling suit by Schrödinger, LLC, New York, NY, 2009. The Protein Data Bank (PDB) contains several AChE complexes with small molecules bound to the active site. We chose the crystal structure of *Torpedo Californica* AChE (PDB code: 1E66) for the virtual screening that was downloaded from the Protein Data Bank (PDB). All water molecules were removed and the protein was prepared in the protein preparation step. A grid which is the representation of shape and properties of receptor using several different sets of fields was also generated. Docking study was carried out using extra precession (SP) mode.

Validation of the docking protocol: Using the flexible ligand docking procedure in Glide-XP, the observed co-crystallographic structure of huprine X from 1E66 was reproduced with a low RMSD value (0.45 Å) indicating that this docking method could be used for predicting the binding modes of other AChE inhibitors. Once the docking protocol was validated, molecules filtered from the databases using pharmacophoric screening were docked in the active site of 1E66 using standard precession (SP) mode.

Biological Activity

Determination of AChE and BuChE inhibitor activity

The Ellman method was applied to test the potency of the compounds in AChE inhibition.[5] GAL, a well characterized inhibitor of AChE, was chosen for comparison. The assay solution consisted of a 0.1 M phosphate buffer, pH 8.0, along with 0.34 mM of 5,5-dithiobis(2-nitrobenzoic acid) (DTNB or Ellman's reagent), 0.02 unit/mL AChE and 0.55 mM acetylthiocholine iodide. The concentration of the inhibitors was 2 μM which is the IC_{50} value of Galanthamine. Initial rate assays were performed at 37°C using a Versamax (Molecular Devices) micro-plate reader. The rate of increase of the absorbance at 412 nm was followed for 15 min. The final assay volume was 150 µL for each well. Assays were also carried out with a blank solution containing all components except AChE, to account for nonenzymatic reaction and with a control solution containing all components including the enzyme but not the inhibitors. Each plate had four parallel set of GAL for validation of the assay. The reaction rates were compared and the percent inhibition due to the presence of test compounds was calculated. Four parallel assays of each inhibitor were performed. The percent inhibition of the enzyme activity due to the

presence of test compound with respect to control was calculated by the following expression:

$[(\nu_0 - \nu_i)/\nu_0]\times100$, where ν_i and ν_0 are the rates calculated in the presence and absence of inhibitor, respectively.

The inhibition of BuChE was carried out using the same assay with minor modifications as follows: The assay solution consisted of 0.1M phosphate buffer pH=8, with 0.34 mM of DTNB, 0.02 unit/mL BuChE and 0.55 mM S-butyrylthiocholine chloride. The concentration of the inhibitors was 10 μM, same as the IC_{50} values of GAL.

Inhibitor concentration dependence studies were also performed with the best compounds to determine their IC_{50} values.[6] For AChE experiments the concentration range of the inhibitors was 0-25 μM, while for BuChE the compounds were tested in the 0-50 μM range.

Inhibition of Aβ fibril formation

The assay was carried out using a standard published procedure.[7] A 40 mg/mL (9.24 mM) solution of the Aβ(1-40) peptide was prepared in 100 mM NaOH. It was diluted in 10 mM HEPES, 100 mM NaCl, 0.02% NaN_3 (pH=7.4) buffer to a final peptide concentration of 100 μM. The inhibitor solutions (10 mM in dimethyl sulfoxide (DMSO)) and were added to the Aβ samples in HEPES buffer in a molar ratio of inhibitor/Aβ=1. The mixture was vortexed for 30 s and the solutions were incubated at 37°C (77 rpm shaking) for four/six days and samples were withdrawn for analysis. The extent of inhibition was determined by Thioflavin-T fluorescence spectroscopy. [8] A Hitachi F-2500 fluorescence spectrophotometer was used to measure the fluorescence.

The incubated peptide solutions were briefly vortexed and 3.5 µl aliquots were withdrawn and added into 700 µL of 5 µM Thioflavin-T prepared freshly in 50 mM glycine-NaOH (pH=8.5) buffer. The maximum fluorescence intensity of these mixtures was determined at 484±5 nm emission wavelength ($\lambda_{excitation}$= 435 nm). For the purposes of a screening assay, the unmodulated fibril signal (control) generated under the conditions of the assay in the presence of 1% DMSO (solvent control) is taken as 100%.

Due to different lots of the synthetic peptide used the fluorescence of the control samples reached saturation at different times and thus, the fibrillogenesis inhibition of the chalcones were measured after 6 days, while that of the coumarins were obtained after 4 days.

Since the compounds may have intrinsic fluorescence we have determined the fluorescence value of the buffer with DMSO, the fluorescence of the compounds in the buffer and the fluorescence intensity of the inhibitor and THT containing solutions.

The data are shown below. Based on the data the compounds did not affect the fluorescence values of the samples.

Table SI 1. Raw fluorescence data of the compounds in buffer and in THT-containing buffer. The fluorescence intensity of the buffer with DMSO (used for dissolution of the inhibitors) was 2.800.

Compounds in buffer (50 mM Glycine pH= 8.5)			Compounds in ThT + buffer (50 mM Glycine pH= 8.5)	
Coumarins			Coumarins	
ThT	6.322		**ThT**	7.297
16	4.744		**16**	7.218
17	2.885		**17**	7.547
18	3.144		**18**	6.728
22	2.898		**22**	6.765
19	2.998		**19**	7.278
20	3.155		**20**	7.561
21	2.75		**21**	7.316
Chalcones			Chalcones	
3	3.067		**3**	7.462
1	2.89		**1**	6.75
2	3.222		**2**	6.978
4	2.868		**4**	7.936
5	3.531		**5**	6.809
6	3.158		**6**	6.946
8	3.266		**8**	7.098
7	3.096		**7**	7.037
9	3.007		**9**	7.156
11	3.084		**11**	7.104
10	2.984		**10**	6.862
12	3.062		**12**	7.125
13	3.648		**13**	6.622
14	2.915		**14**	6.816
15	2.917		**15**	6.959

Atomic Force Microscopy

The morphology of the peptide deposits were studied by atomic force microscopy (AFM).[6] 2 µL Aliquots were placed on freshly cleaved mica sheets and air dried. The buffer salts were washed off with deionized water. The measurements were carried out

using a Bruker-Innova SPM instrument. The images shown in the manuscript were obtained after 6 days of incubation.

Determination of inhibitor activity in Aβ oligomer formation and oligomer disassembly by biotinyl- streptavidin assays[9]

Inhibition of Aβ oligomer assembly Biotinyl-Aβ(1-42) (1 mg/mL) solution in HFIP was dried at -75 ºC, trifluoroacetic acid was added for 10 min at room temperature to disaggregate the peptide. Then the mixture was dissolved in DMSO (500 nM). The monomeric peptide (2μL) was dispensed into a 96-well plate and 100 μL of PBS with the test compound and 1% DMSO was added at ambient temperature to initiate oligomer formation. After 30 min incubation, 50 μL of 0.3% v/v Tween 20 was added to stop oligomer assembly. This mixture (50 μL) was then assayed for oligomer content.

Biotinyl-Aβ(1-42) single-site streptavidin-based assay for measurement of biotinyl-Aβ(1-42) oligomer content NeutrAvidin (50 μl, 1 μg/mL NA in 10mM NaPi (pH=7.5)) was coated per well at 4 °C overnight on sealed Costar 9018 high-binding ELISA plates. The plates were blocked by 200 μl PBS, 10mM sodium phosphate, 150mM NaCl (pH 7.5), 0.1%v/v Tween 20 at ambient temperature for 1–2 h and stored at 4 °C. The blocking solution was removed and the sample (50 μl containing up to 10 nM biotinyl-Aβ) was allowed to bind for 2 h at room temperature (RT). The wells were washed three times with TBST (20mM Tris–HCl, 34mM NaCl (pH=7.5) and 0.1% v/v Tween 20) on a Biotek EL x 50 plate washer. Then, 50 μL of 1:20,000 SA–HRP in PBS + 0.1% v/v Tween 20 was added, the sealed plate was incubated for another 1 h (RT). After incubation, the plate was washed with TBST and 100 μL of tetramethylbenzidine/H_2O_2 substrate solution was added, and the plate was incubated at RT for 5–10min. The

reaction was stopped 100 µl of 1% (v/v) H_2SO_4 and the OD_{450nm} was measured on a Biotech Synergy HT plate reader. The oligomer signal generated under the conditions of the assay in the presence of 1% DMSO (solvent control) and absence of compound was taken as 100%.

The IC_{50} data were determined as described earlier.[6]

Determination of Aβ oligomer disassembly activity

Preparation of pre-formed biotinyl-Aβ(1-42) oligomers

Biotinyl-Aβ(1-42) was disaggregated as described above. DMSO was added to the dried film to produce an 8 µg bio42/ml stock solution. The solution was diluted 50-fold into PBS (20 mM sodium phosphate, 145 mM NaCl, pH 7.5) in a polypropylene container to 33.7 nM monomer (0.16 µg/ml) after ten minutes. After one hour at RT an equal volume of PBS + 0.6% v/v Tween 20 was added to stop further oligomer formation and stabilize the oligomers. The oligomers are >70 kDa, and their size distribution by size exclusion chromatography is similar to that of Aβ oligomers from AD brain.

Oligomer dissociation assay

Preformed bio42 oligomers (25 µl, 16.8 nM) in PBS + 0.3% Tween 20 were added into a wide well 96-well plate (Fisher 12565502) followed by 125 µl of PBS with the disassembly agent and 1 % v/v DMSO. The plate was sealed (Nunc 236366) and shaken (150 rpm) at RT for 16-18 hours. The remaining amount of oligomers was measured by adding 100 µl from each well to an NA-coated (50 ng/well) wells of a Costar 9018 ELISA plate and measured as described above.

The IC_{50} data were determined as described earlier.[6]

Spectroscopic characterization of the products

(E)-1-(3-((diethylamino)methyl)phenyl)-3-phenylprop-2-en-1-one (**1**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.98 (s, 1H, Ar –H), 7.91-7.89 (m, 1H, Ar –H). 7.85-7.79 (d, 1H, -CH=CH, *J* = 15.9 Hz), 7.67-7.61 (m, 2H, Ar –H), 7.58-7.53 (d, 1H, -CH=CH, *J*= 15.6 Hz), 7.48-7.39 (m, 5H, Ar –H), 3.65 (s, 2H, $-CH_2$), 2.59-2.52 (q, 4H, $-CH_2$, *J*= 7.2 Hz), 1.08-1.04 (t, 6H, $-CH_3$, *J*= 7.0 Hz) ;

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.6, 144.6, 138.1, 134.8, 133.3, 130.4, 128.9, 128.7, 128.4, 127.0, 122.1, 57.2, 46.6, 11.6;

MS- $C_{20}H_{23}NO$ (293), m/z (%): 293 (M)

(E)-3-phenyl-1-(3-((4-phenylpiperazin-1-yl)methyl)phenyl)prop-2-en-1-one (**2**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.96 (s, 1H, Ar –H), 7.87-7.89 (m, 1H, Ar –H), 7.84-7.78 (d, 1H, CH=CH, *J*= 15.6 Hz), 7.64-7.62 (m, 2H, Ar –H), 7.56-7.50 (m, 2H, Ar –H, 1H, CH=CH), 7.46-7.29 (m, 4H, Ar H), 7.31-7.25 (m, 5H, Ar –H), 3.60 (s, 2H, $-CH_2$), 3.53 (s, 2H, $-CH_2$), 2.59-2.51 (m, 8H, $-CH_2$);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.0, 144.3, 138.2, 137.6, 137.2, 134.3, 133.4, 133.1, 130.0, 128.8, 128.6, 128.4, 128.0, 127.9, 127.7, 126.8, 126.6, 126.5, 121.6, 62.4, 52.4

(E)-1-(3-((diethylamino)methyl)phenyl)-3-(naphthalen-2-yl)prop-2-en-1-one (**3**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 8.02-8.00 (m, 2H, Ar –H), 7.95-7.79 (m, 4H, Ar –H, 1H, CH=CH), 7.68 (s, 1H, -Ar –H), 7.63-7.59 (m, 1H, -CH-CH), 7.53-7.44 (m, 2H, Ar -H), 3.67 (s, 2H, $-CH_2$), 2.60-2.53 (q, 4H, $-CH_2$, *J*= 7.2 Hz), 1.09-1.04 (t, 6H, $-CH_3$, *J*= 7.2 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.6, 144.7, 140.5, 138.2, 134.3, 133.4, 133.3, 132., 128.8, 128.7, 128., 128.5, 127.7, 127.3, 127.0, 126.7, 123.6, 122.3, 57.2, 46.6, 11.6;

MS - $C_{24}H_{25}NO$ (343), m/z (%): 344 (M+1)

(E)-1-(3-((diethylamino)methyl)phenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**4**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 8.02 (s, 1H, Ar –H), 7.92-7.89 (d, 1H, Ar –H, *J*= 7.8 Hz), 7.80-7.75 (d, 1H, CH=CH, *J*=15.6 Hz), 7.60-7.58 (d, 1H, Ar –H, *J*= 7.2 Hz), 7.47-7.42 (m, 1H, Ar –H, 1H, CH=CH), 7.26-7.20 (m, 2H, Ar –H), 6.91-6.88 (d, 1H, Ar –H, *J* = 8.1 Hz), 3.96 (s, 3H, -OCH_3), 3.93 (s, 3H, -OCH_3), 3.68 (s, 2H, -CH_2), 2.61-2.54 (q, 4H, -CH_2, *J*= 7.2 Hz), 1.10-1.05 (t, 6H, -CH_3, *J*= 7.2 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.5, 151.2, 149.2, 144.7, 138.3, 133.1, 128.7, 128.3, 127.7, 126.9, 123.1, 120.0, 110.9, 109.9, 57.1, 55.8, 4.5, 11.4

(E)-1-(3-((4-benzylpiperazin-1-yl)methyl)phenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**5**)

^{1}H-NMR ($CDCl_3$, 499.702 MHz) δ: 7.97 (s, 1H, Ar –H), 7.91-7.89 (d, 1H, Ar –H, *J*= 7.5 Hz), 7.78-7.74 (d, 1H, CH=CH, *J*= 15.5 Hz), 7.55-7.54 (d, 1H, Ar –H, *J*= 7.5 Hz), 7.46-7.44 (d, 1H, Ar –H), 7.42-7.39 (d, 1H, CH=CH, *J*= 15.5 Hz), 7.31-7.30 (m, 4H, Ar –H), 7.27-7.22 (m, 2H, Ar –H), 7.18-7.17 (m, 1H, Ar –H), 6.91-6.89 (d, 1H, CH=CH, *J*= 8 Hz), 3.95 (s, 3H, -OCH_3), 3.929 (s, 3H, -OCH_3), 3.62 (s, 2H, -CH_2), 3.54 (s, 2H, -CH_2), 2.54 (bs, 8H, -CH_2);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.5, 151.3, 149.1, 144.9, 138.4, 133.4, 129.2, 129.1, 128.4, 128.1, 127.7, 127.3, 127.1, 123.2, 120.0, 110.9, 109.9, 62.8, 62.4, 55.9, 52.7

(E)-1-(3-((diethylamino)methyl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**6**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.94 (s, 1H, Ar –H), 7.87-7.85 (d, 1H, Ar –H, *J* = 8.1 Hz), 7.79-7.74 (d, 1H, CH=CH, *J* = 15.6 Hz), 7.60-7.55 (m, 2H, Ar –H, 1h, CH=CH), 7.44-7.41 (m, 1H, Ar –H), 7.40-7.24 (m, 1H, Ar –H), 6.92-6.90 (d, 1H, Ar –H, *J* = 6.9 Hz), 3.83 (s, 3H, -OCH_3), 3.63 (s, 2H, -CH_2), 2.57-2.50 (q, 4H, -CH_2, *J* = 7.2 Hz), 1.06-1.02 (t, 6H, -CH_3, *J* = 7.2 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.7, 161., 144.5, 138.4, 133.1, 130.2, 128.7, 128.4, 127.6, 126.9, 119.9, 114.3, 57.3, 55.3, 46.6, 11.6;

MS- $C_{21}H_{25}NO_2$ (323), m/z (%): 323 (M)

(E)-3-(4-methoxyphenyl)-1-(3-((4-phenylpiperidin-1-yl)methyl)phenyl)prop-2-en-1-one (**7**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.98 (s, 1H, Ar –H), 7.93-7.90 (d, 1H, Ar –H, *J* = 7.5 Hz), 7.83-7.77 (d, 1H, CH=CH, *J* = 15.6 Hz), 7.63-7.59 (m, 3H, Ar –H), 7.49-7.41 (m, 1H, Ar –H, 1H, CH=CH), 7.32-7.19 (m, H, Ar –H), 6.96-6.93 (d, 1H, Ar –H, *J* = 7.2 Hz), 3.85 (t, 3H, -OCH_3), 3.63 (s, 2H, -CH_2), 3.04-3.00 (m, 2H, -CH_2), 2.49-2.51 (p, 1H, -CH, J_1= 13.3 Hz, J_2= 7.0 Hz), 2.17-2.08 (m, 2H, Ar –H), 1.84-1.81 (m, 4H, -CH_2);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.7, 161.6, 146.3, 144.5, 139.0, 138.4, 133.4, 130.2, 129.0, 128.4, 128.3, 127.5, 127.1, 126.8, 126.0, 119.9, 114.3, 63.1, 55.3, 54.2, 42.5, 33.4

(E)-1-(3-((4-benzylpiperazin-1-yl)methyl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**8**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.94 (s, 1H, Ar –H), 7.90-7.88 (d, 1H, Ar –H, *J* = 7.2 Hz), 7.81-7.76 (d, 1H, CH=CH, *J* = 15.6 Hz), 7.62-7.59 (d, 2H, Ar –H, *J* = 9 Hz), 7.53 (m, 1H, Ar –H), 7.46-7.42 (m, 1H, Ar –H), 7.43-7.39 (m, 1H, Ar –H), 7.31-7.25 (m, 4H, Ar –H), 6.95-6.92 (d, 1H, Ar –H, *J* = 8.7 Hz), 3.85 (s, 3H, -OCH_3), 5.59 (s, 2H, -CH_2), 3.52 (s, 2H, -CH_2), 2.51 (bs, 8H, -CH_2);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.5, 161.6, 144.6, 138.6, 133.3, 130.2, 129.2, 129.0, 128.4, 128.1, 127.5, 127.2, 127.0, 119.7, 114.3, 62.9, 62.6, 55.3, 52.9

(E)-1-(3-((diethylamino)methyl)phenyl)-3-(3-methoxyphenyl)prop-2-en-1-one (**9**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.97 (s, 1H, Ar –H), 7.90-7.88 (d, 1H, Ar –H, *J* = 6.6 Hz), 7.80-7.52 (d, 1H, CH=CH, *J* = 15.6 Hz), 7.61-7.59 (d, 1H, Ar –H, *J* = 7.5 Hz), 7.55 (s, 1H, Ar –H), 7.50-7.43 (d, 1H, CH=CH, *J* = 14.4 Hz), 7.37-7.31 (m, 1H, Ar –H), 7.2-7.23 (m, 1H, Ar –H), 7.17-7.1 (m, 1H, Ar –H), 6.98-6.96 (d, 1H, Ar –H, *J* = 7.8 Hz), 3.85 (s, 3H, -OCH_3), 3.66 (s, 2H, -CH_2), 2.59-2.52 (q, 4H, -CH_2, *J* = 7.2 Hz), 1.09-1.04 (t, 6H, -CH_3, *J* = 7.2 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.7, 161.6, 144.5, 138.4, 133.1, 130.2, 128.7, 128.4, 127.6, 126.9, 119.9, 114.3, 57.3, 55.4, 46.6, 11.6

(E)-3-(3-methoxyphenyl)-1-(3-((4-phenylpiperidin-1-yl)methyl)phenyl)prop-2-en-1-one (**10**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.99 (s, 1H, Ar –H), 7.93-7.91 (d, 1H, Ar –H, *J* = 6.6 Hz), 7.81-7.76 (d, 1H, -CH=CH, *J* = 15.6 Hz), 7.62-7.5 (m, 2H, Ar –H), 7.51-7.47 (m, 1H, Ar –H), 7.43-7.23 (m, 8H, Ar –H, 1H, -CH=CH), 6.98-6.95 (d, 1H, Ar –H), 3.82 (s, 3H, $-OCH_3$), 3.63 (s, 2H, $-CH_2$), 3.09-2.98 (m, 2H, $-CH_2$), 2.53-2.48 (p, 1H, -CH, J_1= 13.1 Hz, J_2= 6.9 Hz), 2.17-2.06 (m, 2H, $-CH_2$), 1.83-1.81 (m, 4H, $-CH_2$);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.5, 159.8, 146.2, 144.6, 139.0, 138.1, 136.2, 133.9, 133.6, 129.9, 129.1, 128.9, 128.5, 128.4, 127.2, 127.1, 126.8, 126.6, 126.5, 122.4, 121.1, 116.2, 113.3, 62.9, 55.3, 54.1, 42.4, 33.3

(E)-3-(3-methoxyphenyl)-1-(3-((4-phenylpiperazin-1-yl)methyl)phenyl)prop-2-en-1-one (**11**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.95 (s, 1H, Ar –H), 7.91-7.89 (d, 1H, Ar –H, *J* = 6.3 Hz), 7.79-7.74 (d, 1H, -CH=CH, *J* = 15.6 Hz), 7.57-7.54 (m, 1H, Ar –H), 7.48-7.42 (m, 1H, Ar –H, 1H, -CH=CH), 7.33-7.25 (m, 7H, Ar –H), 7.16 (m, 1H, Ar –H), 6.98-6.96 (d, 1H, Ar –H), 3.84 (s, 3H, $-OCH_3$), 3.59 (s, 2H, $-CH_2$), 3.52 (s, 2H, $-CH_2$), 2.50 (bs, 8H, $-CH_2$);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.5, 159.8, 144.6, 138.8, 138.1, 137.8, 136.2, 133.6, 129.9, 129.2, 128.5, 127.2, 122.4, 121.0, 116.2, 113.4, 62.9, 62.5, 55.3, 52.9, 52.8

(E)-1-(3-((diethylamino)methyl)phenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**12**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.97 (s, 1H, Ar –H), 7.90-7.88 (d, 1H, -CH=CH, *J* = 15.6 Hz), 7.66-7.61 (m, 3H, Ar –H), 7.51-7.45 (m, 1H, Ar –H, 1H, CH=CH, *J* = 15.9 Hz), 7.14-7.08 (t, 2H, Ar –H, *J* = 8.7 Hz), 3.65 (s, 2H, $-CH_2$), 2.59-2.52 (q, 4H, $-CH_2$, *J* = 6.9 Hz), 1.09-1.04 (t, 6H, $-CH_3$, *J* = 7.2 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.4, 162.3, 143.3, 140.7, 138.0, 133.4, 131.1, 130.4, 130.3, 128.7, 128.5, 126.9, 121.9, 121.8, 116.2, 115.9, 57.3, 46.6, 11.6;

MS- $C_{20}H_{22}FNO$ (311), m/z (%): 311 (M)

(E)-3-(4-chlorophenyl)-1-(3-((diethylamino)methyl)phenyl)prop-2-en-1-one (**13**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.89 (s, 1H, Ar –H), 7.82-7.80 (d, 1H, Ar –H, *J* = 7.5 Hz), 7.71-7.66 (d, 1H, -CH=CH, *J* = 15.6 Hz), 7.54-7.47 (m, 3H, Ar –H), 7.42-7.31 (m, 3H, Ar –H, 1H, -CH=CH), 3.57 (s, 2H, $-CH_2$), 2.51-2.44 (q, 4H, $-CH_2$, *J* = 7.2 Hz), 1.00-0.96 (t, 6H, $-CH_3$, *J* = 7.2 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.3, 143.1, 140.7, 137.9, 136.3, 133.5, 129.6, 129.2, 128.7, 128.5, 127.0, 122.6, 57.3, 46.8, 11.6

(E)-3-(4-bromophenyl)-1-(3-((diethylamino)methyl)phenyl)prop-2-en-1-one (**14**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.97 (s, 1H, Ar –H), 7.90-7.88 (d, 1H, Ar –H, *J* = 7.5 Hz), 7.77-7.72 (d, 1H, -CH=CH, *J* = 15.6 Hz), 7.62-7.27 (m, 6H, Ar –H, 1H, -CH=CH), 2.59-2.52 (q, 4H, $-CH_2$, *J* = 7.2 Hz), 1.09-1.04 (t, 6H, $-CH_3$, *J* = 7.2 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 190.3, 143.2, 140.7, 137.9, 133.8, 133.5, 132.1, 129.8, 128.7, 128.3, 127.0, 124.7, 122.7, 57.3, 46.7, 11.6

(E)-3-([1,1'-biphenyl]-4-yl)-1-(3-((diethylamino)methyl)phenyl)prop-2-en-1-one (**15**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 7.99 (s, 1H, Ar –H), 7.92-7.90 (d, 1H, Ar -H, *J* = 7.5 Hz), 7.89-7.84 (1H, -CH=CH, *J* = 15.9 Hz), 7.75-7.72 (d, 2H, Ar –H, *J* = 8.4 Hz), 7.68-7.56 (m, 5H, Ar –H, 1H, -CH=CH), 7.49-7.25 (4H, Ar –H), 2.59-2.52 (q, 4H, $-CH_2$, *J* = 7.2 Hz), 1.09-1.04 (t, 6H, $-CH_3$, *J* = 7.2 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 119.1, 144.6, 143.6, 141.2, 140.5, 138.6, 134.3, 133.8, 129.4, 129.3, 129.2, 128.9, 128.3, 127.9, 127.4, 122.5, 57.7, 47.1, 12.1

3-acetyl-7-methoxy-2H-chromen-2-one

^{1}H-NMR ($CDCl_3$, 499.702 MHz) δ: 8.52 (s, 1H, -CH=C), 7.82-7.80 (d, 1H, Ar –H, *J* = 9 Hz), 7.01-6.99 (dd, 1H, Ar –H, J_1 = 2.5 Hz, J_2 = 6.5 Hz), 6.95-6.94 (d, 1H, Ar –H, *J* = 2 Hz), 3.98 (s, 3H, $-OCH_3$), 2.58 (s, 3H, $-CH_3$)

3-(2-(4-benzylpiperazin-1-yl)acetyl)-7-methoxy-2H-chromen-2-one (**16**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 8.52 (s, 1H, -CH=C), 7.57-7.54 (d, 1H, Ar –H, J = 9 Hz), 7.33-7.27 (m, 5H, Ar –H), 6.93-6.89 (dd, 1H, Ar –H, J_1 = 2.1 Hz, J_2 = 6.3 Hz), 6.84-6.83 (d, 1H, Ar –H, J = 2.1 Hz), 3.96 (s, 2H, -CH_2), 3.92 (s, 3H, -OCH_3), 3.52 (s, 2H, -CH_2), 2.63-2.52 (m, 8H, -CH_2);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 199.4, 165.2, 157.6, 148.0, 138.0, 131.4, 129.2, 128.2, 126.9, 113.9, 100.2, 67.3, 63.0, 56.0, 53.4, 52.9;

MS- $C_{23}H_{24}N_2O_4$ (392), m/z (%): 391 (M-1)

7-methoxy-3-(2-(4-phenylpiperidin-1-yl)acetyl)-2H-chromen-2-one (**17**)

^{1}H-NMR ($CDCl_3$, 499.702 MHz) δ: 8.54 (s, 1H, -CH=C), 7.58-7.55 (d, 1H, Ar –H, J = 9 Hz), 7.30-7.19 (m, 5H, Ar –H), 6.93-6.89 (dd, 1H, Ar –H, J_1 = 2.4 Hz, J_2 = 6.3 Hz), 6.85-6.84 (d, 1H, Ar –H, J = 2.4 Hz), 3.99 (s, 2H, -CH_2), 3.92 (s, 3H, -OCH_3), 3.15-3.11 (m, 2H, -CH_2), 2.56-2.48 (m, 1H, -CH_2), 2.31-2.23 (m, 2H, -CH_2), 1.98-1.74 (m, 3H, -CH_2);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 194.9, 165.6, 158.0, 148.4, 146.7, 131.8, 128.8, 127.3, 126.5, 114.3, 112.5, 100.7, 68.1, 56.4, 55.0, 42.8, 33.7;

MS- $C_{23}H_{23}NO_4$ (377), m/z (%): 378 (M+1)

7-methoxy-3-(2-(4-phenylpiperazin-1-yl)acetyl)-2H-chromen-2-one (**18**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 8.47 (s, 1H, -CH=C), 7.50-7.47 (d, 1H, Ar –H, J = 9 Hz), 7.21-7.15 (m, 2H, Ar –H), 6.88-6.82 (m, 4H, Ar –H), 6.77-6.76 (m, 2H, Ar –H), 3.95 (s, 2H, -CH_2), 3.85 (s, 3H, -OCH_3), 3.21-3.17 (m, 4H, -CH_2), 2.79-2.69 (m, 4H, -CH_2););

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 194.1, 165.3, 159.4, 157.7, 151.3, 148.0, 131.4, 129.0, 120.2, 119.6, 116.0, 113.9, 112.0, 100.3, 67.1, 56.0, 53.4, 49.0, 29.7;

MS- $C_{22}H_{22}N_2O_4$ (378), m/z (%): 379 (M+1)

7-methoxy-3-(2-(4-(pyridin-2-yl)piperazin-1-yl)acetyl)-2H-chromen-2-one (**19**)

^{1}H-NMR ($CDCl_3$, 499.702 MHz) δ: 8.55 (s, 1H, -CH=C), 8.19-8.18 (dd, 1H, Ar –H), 7.58-7.56 (d, 1H, Ar –H, *J* = 9 Hz), 7.49-7.46 (m, 1H, Ar –H), 6.93-6.90 (dd, 1H, Ar –H), 6.84-6.83 (d, 1H, Ar –H, *J* = 2.5 Hz), 6.66-6.64 (d, 1H, Ar –H, *J* = 8 Hz), 6.63-6.60 (m, 1H, Ar –H), 4.02 (s, 2H, $-CH_2$), 3.92 (s, 3H, $-OCH_3$), 3.62-3.60 (t, 4H, $-CH_2$, *J* = 4.5 Hz), 2.74-2.72 (t, 4H, $-CH_2$, *J* = 5 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 194.1, 165.3, 159.4, 157.6, 149.6, 148.2, 147.9, 137.4, 131.5, 113.9, 112.0, 107.0, 100.3, 67.3, 56.0, 53.2, 45.0;

MS- $C_{21}H_{21}N_3O_4$ (379), m/z (%): 380 (M+1).

7-methoxy-3-(2-(4-(pyrimidin-2-yl)piperazin-1-yl)acetyl)-2H-chromen-2-one (**20**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 8.56 (s, 1H, -CH=C), 8.31-8.29 (d, 2H, Ar –H, *J* = 4.8 Hz), 7.59-7.56 (d, 1H, Ar –H, *J* = 8.7 Hz), 6.94-6.90 (dd, 1H, Ar –H, J_1 = 9 Hz, J_2 = 8.7 Hz), 6.85-6.84 (d, 1H, Ar –H, *J* = 2.1 Hz), 6.49-6.47 (t, 1H, Ar –H, *J* = 4.8 Hz), 4.01 (s, 2H, $-CH_2$), 3.92-3.88 (m, 3H, $-OCH_3$, 4H, $-CH_2$), 2.69-2.66 (t, 4H, $-CH_2$, *J* = 5.1 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 194.1, 165.3, 157.7, 148.2, 131.5, 120.1, 117.2, 113.9, 112.0, 109.8, 100.2, 67.3, 56.0, 53.3, 43.5;

MS- $C_{20}H_{20}N_4O_4$ (380), m/z (%): 381 (M+1)

7-methoxy-3-(2-morpholinoacetyl)-2H-chromen-2-one (**21**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 8.54 (s, 1H, -CH=C), 7.58-7.55 (d, 1H, Ar –H, *J* = 8.7 Hz), 6.94-6.90 (dd, 1H, Ar –H, J_1 = 2.4 Hz, J_2 = 6 Hz), 6.85-6.84 (d, 1H, Ar –H, *J* = 2.4 Hz), 3.96 (s, 2H, $-CH_2$), 3.93 (s, 3H, $-OCH_3$), 3.79-3.76 (t, 4H, $-CH_2$, *J* = 4.6 Hz), 2.64-2.61 (t, 4H, $-CH_2$, *J* = 4.3 Hz);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 194.0, 165.4, 148.2, 131.5, 113.9, 112.0, 100.3, 67.6, 66.8, 56.0, 53.8;

MS- $C_{16}H_{17}NO_5$ (303), m/z (%): 304 (M+1)

*3-(2-(4-benzylpiperidin-1-yl)acetyl)-7-methoxy-2H-chromen-2-on*e (**22**)

^{1}H-NMR ($CDCl_3$, 300.126 MHz) δ: 8.50 (s, 1H, -CH=C), 7.56-7.53 (d, 1H, Ar-H, J= 8.7 Hz), 7.29-7.25 (m, 2H, Ar –H), 7.19-7.13 (m, 2H, Ar –H), 6.92-6.88 (dd, 1H, Ar –H, J_1= 2.4 Hz, J_2= 6.3 Hz), 6.84-6.83 (d, 1H, Ar –H, J= 2.1 Hz), 3.91 (s, 3H, -OCH_3, 2H, -CH_2), 2.99-2.95 (m, 2H, -CH_2), 2.54-2.52 (d, 2H, -CH_2, J= 6.3 Hz), 2.10-2.03 (t, 2H, -CH_2, J= 10.5 Hz), 1.64-1.39 (m, 1H, -CH, 4H, -CH_2);

^{13}C-NMR ($CDCl_3$, 75.467 MHz) δ: 194.6, 165.2, 147.8, 140.7, 131.3, 129.1, 128.1, 125.7, 117.2, 113.8, 100.2, 67.7, 56.0, 54.2, 43.2, 37.6, 32.0;

MS- $C_{24}H_{25}NO_4$ (391), m/z (%): 392 (M+1)

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