Supporting Information

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Synthesis and bioactivity of 1-substituted tetrahydroisoquinolines derived from phenolic aldehydes

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S.1: General experimental procedures

All chemicals were obtained from commercial suppliers and, where necessary, purified, in accordance with the instructions of Armarego and Chai[1]. Evaluation of the purity of the obtained derivatives was checked by thin-layer chromatography. Visualization and detection of the chromatogram was achieved using a UV lamp and 0.2% solution of ninhydrin in EtOH. Melting points were determined on a Kofler apparatus. The elemental analysis of the synthesized derivatives was determined by burning the sample in the O_2 atmosphere with He as a carrier (PerkinElmer CHNS/O Analyzer 2400 Series II). Infrared spectra were recorded using the KBr pellet method in the range of 4000-400 cm^{-1} (PerkinElmer BX FTIR). Nuclear magnetic resonance of ^{1}H and ^{13}C nuclei was recorded in DMSO-d_6 or D_2O (Bruker AVANCE Ultrashield 500 plus). All shifts in NMR spectra are given in ppm (δ) relative to TMS as an internal standard or calibrated against the residual signal of DMSO-d_6 (δ_H q, 2.50; δ_C sept, 39.52). The values of coupling constants (*J*) are expressed in Hz. High-resolution mass spectra were recorded by electrospray ionization in positive mode (Agilent 6224 TOF mass spectrometer). All spectrophotometric measurements were performed on a PerkinElmer Lambda 25 UV-Vis instrument.

S.2: Synthesis of 1-substituted THIQs

The reaction mixture containing dopamine hydrochloride (2 mmol) and phenolic aldehyde (2.2 mmol) in 10 mL of the mixture CH_3CN and phosphate buffer (0.1 M, pH 6.5) was incubated at 50°C for 12 hours[2]. After removal of CH_3CN under reduced pressure, the THIQs were extracted with *n*-BuOH (3×15 mL). The organic extract was washed with water (3×5 mL), dried over anhydrous K_2CO_3 and evaporated to dryness. The solid residue was washed with Et_2O and recrystallized from 1M HCl at 4°C. The purity of prepared THIQs was checked by chromatography on a thin layer of silica gel 60 F_{254} (*n*-BuOH-HOAc-H_2O, 4:1:1).

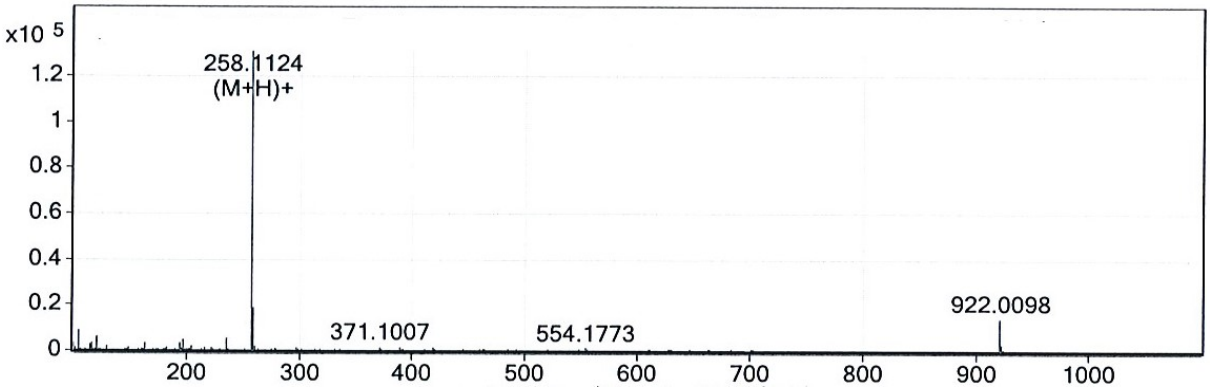

Figure S1: HR-ESI-MS spectrum of **1**.

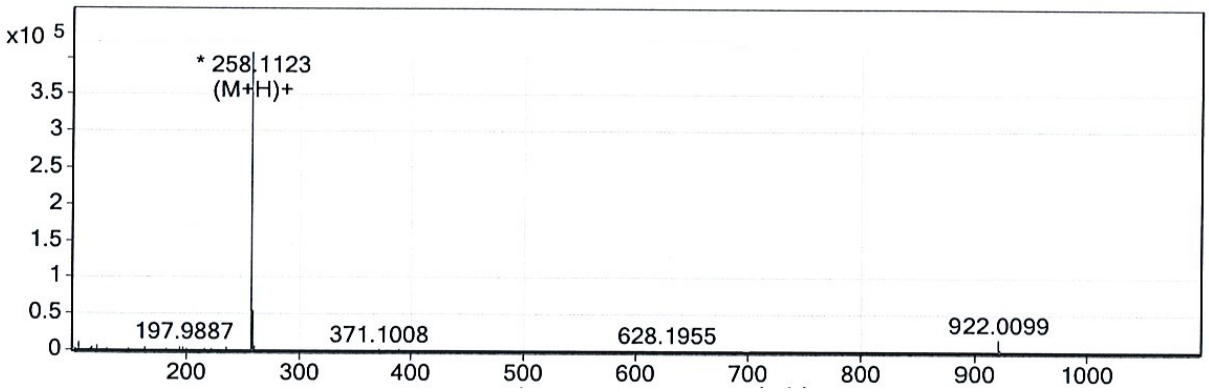

Figure S2: HR-ESI-MS spectrum of **2**.

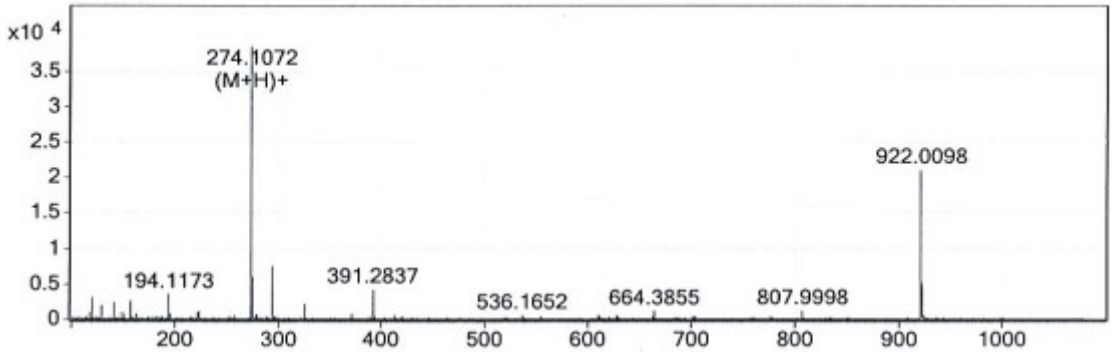

Figure S3: HR-ESI-MS spectrum of **3**.

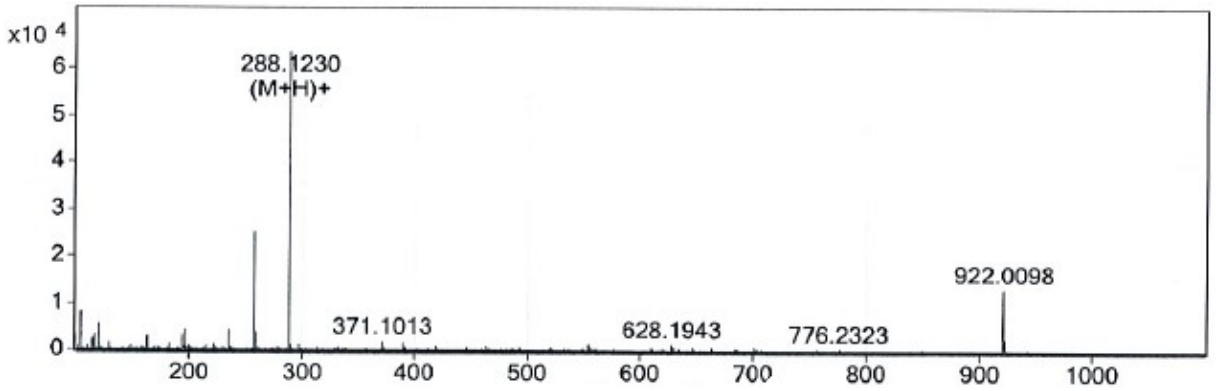

Figure S4: HR-ESI-MS spectrum of **4**.

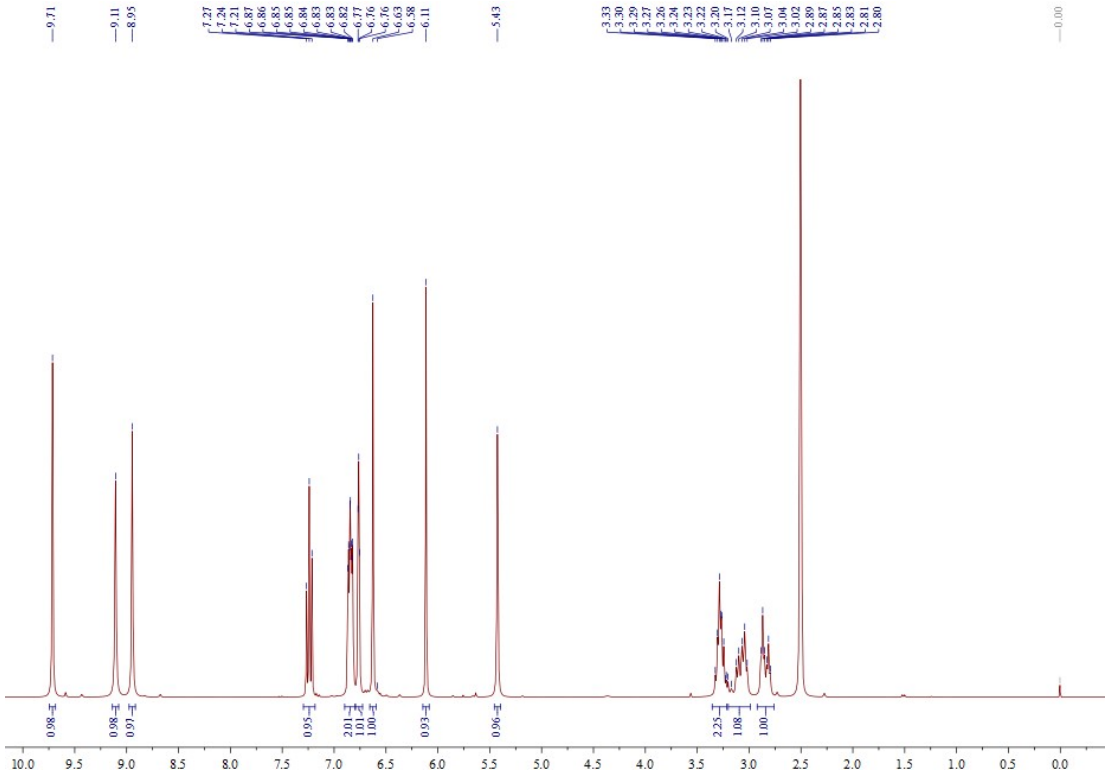

Figure S5: [1]H-NMR (500 MHz, DMSO-d_6) spectrum of **1**.

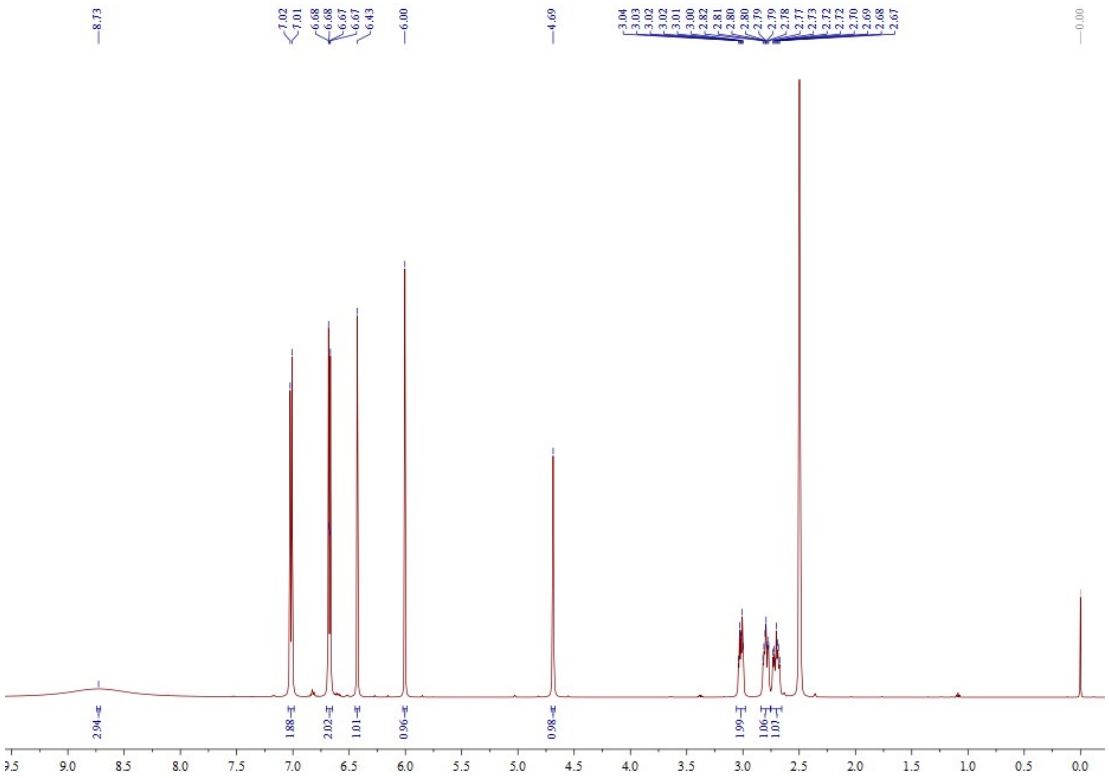

Figure S6: [1]H-NMR (500 MHz, DMSO-d_6) spectrum of **2**.

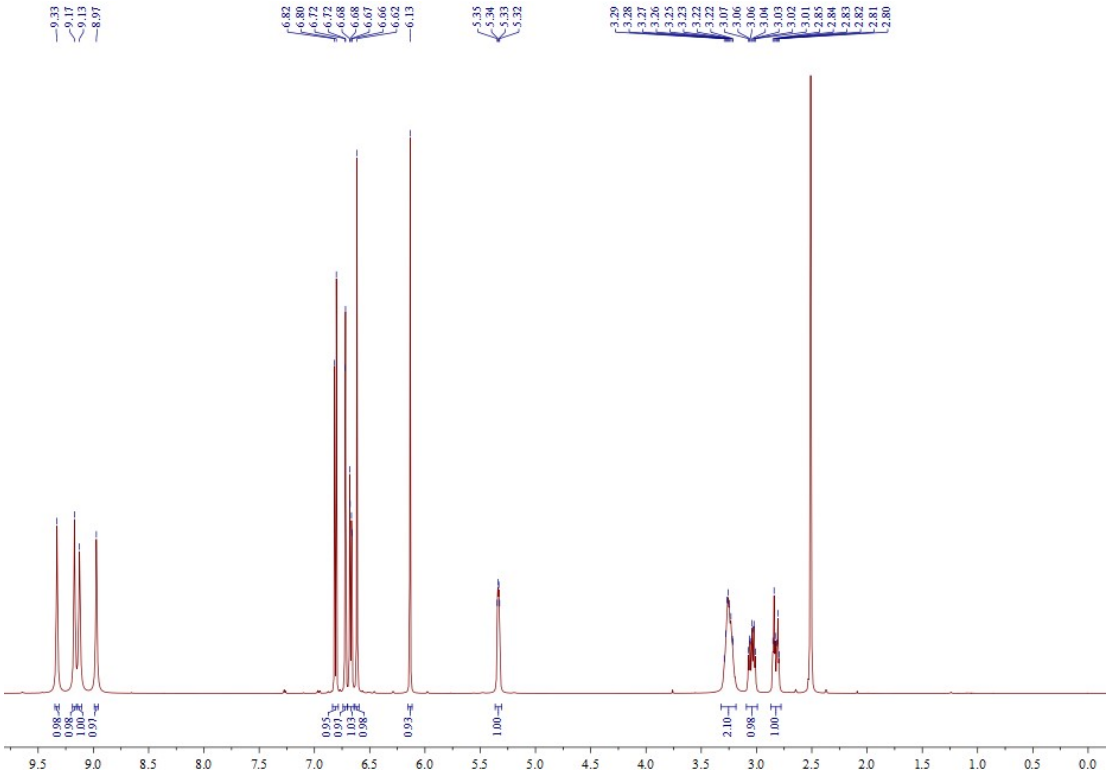

Figure S7: [1]H-NMR (500 MHz, DMSO-d_6) spectrum of **3**.

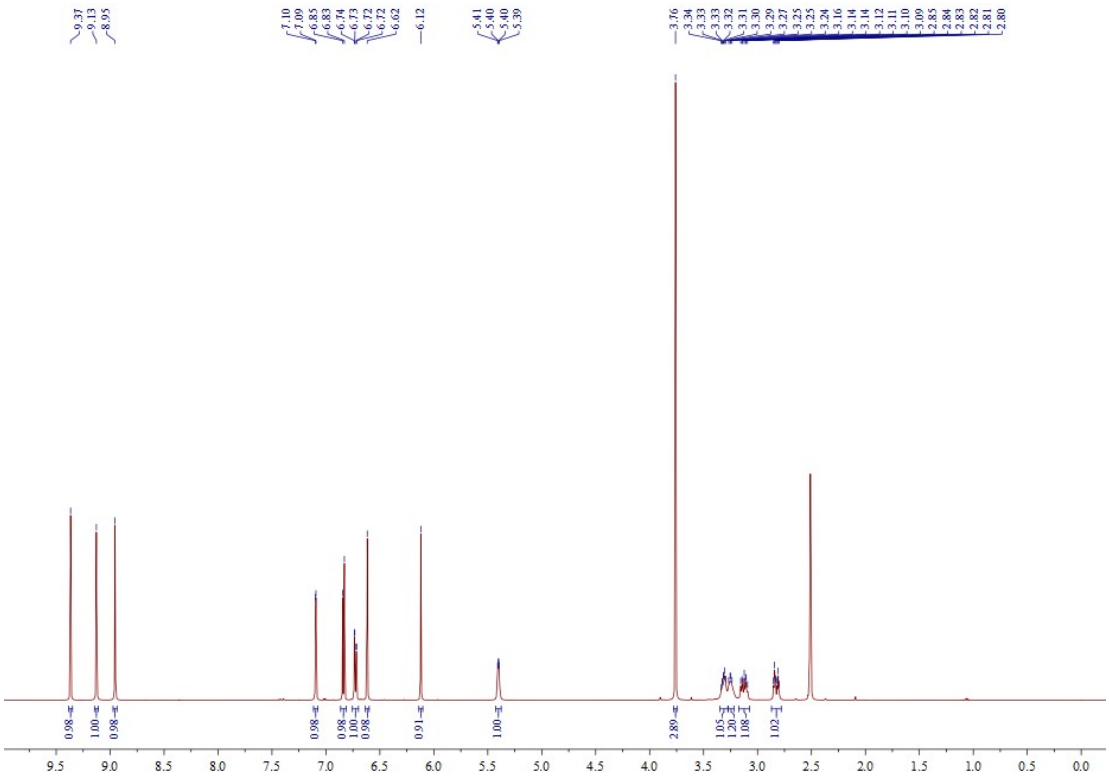

Figure S8: [1]H-NMR (500 MHz, DMSO-d_6) spectrum of **4**.

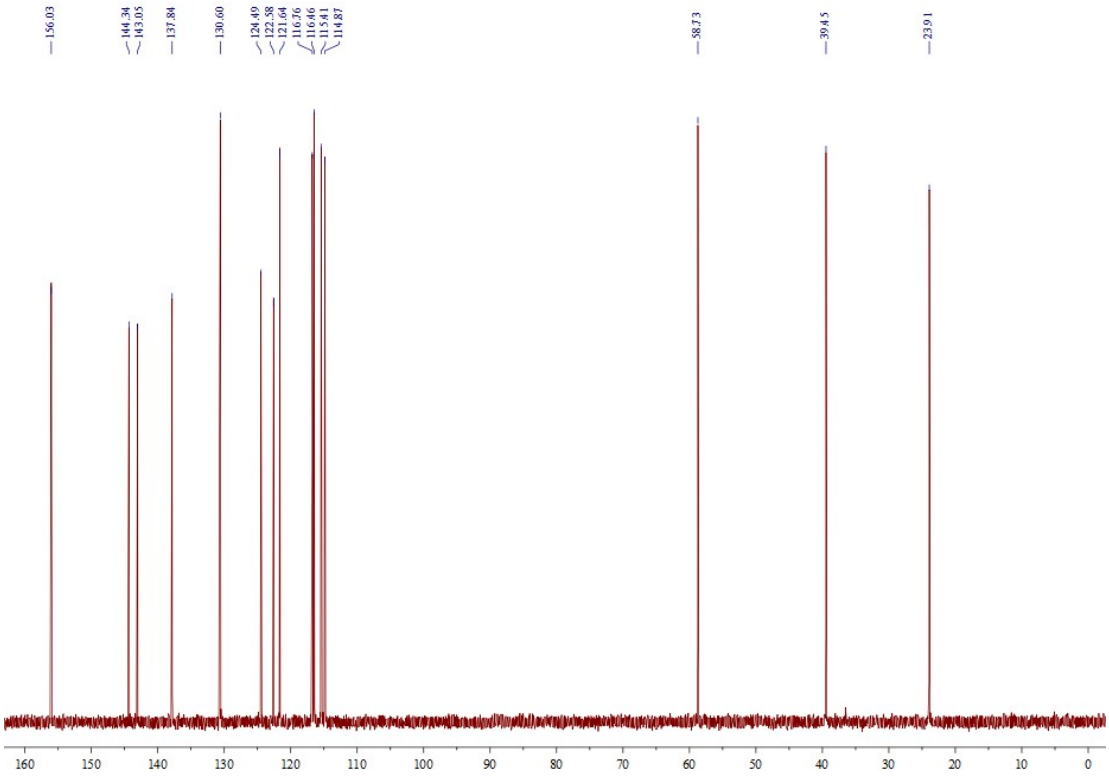

Figure S9: ^{13}C-NMR (500 MHz, D_2O) spectrum of **1**.

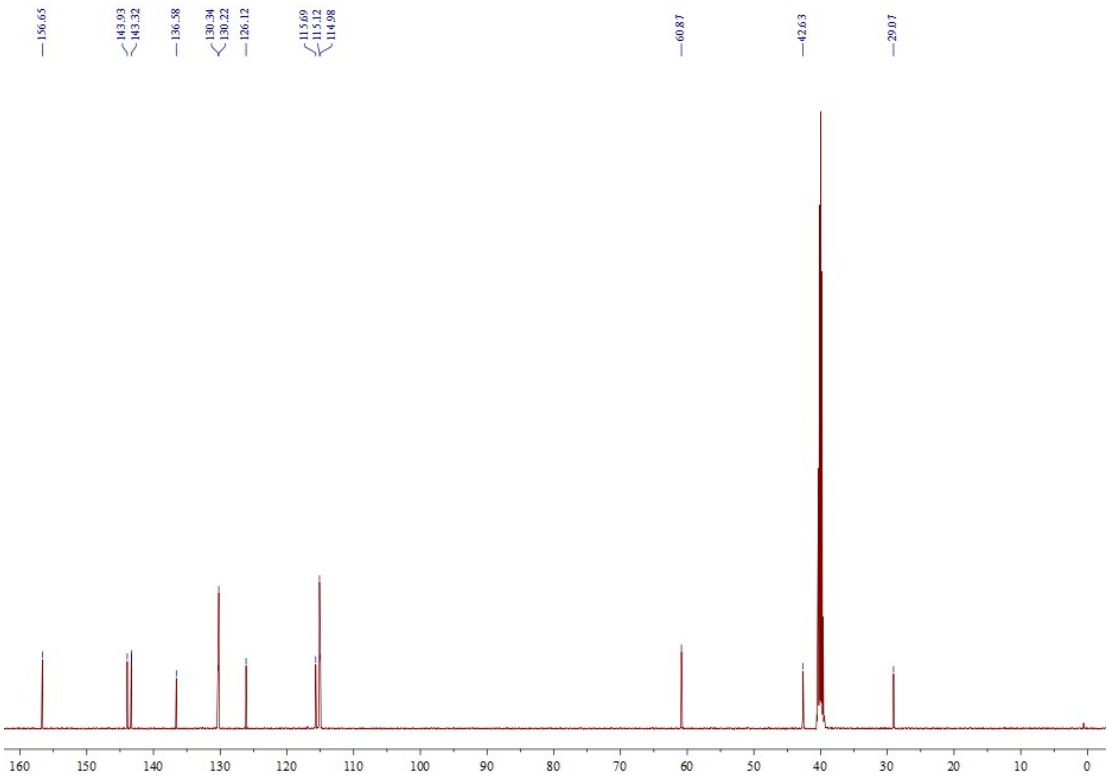

Figure S10: ^{13}C-NMR (125 MHz, DMSO-d_6) spectrum of **2**.

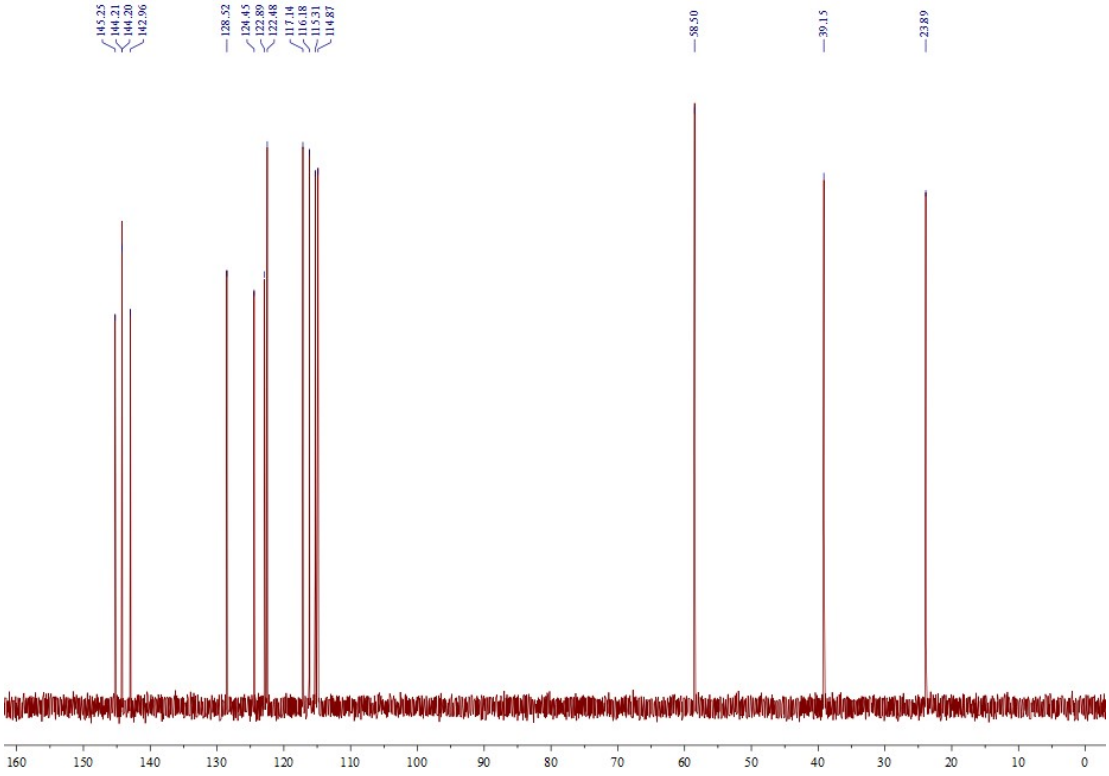

Figure S11: ^{13}C-NMR (125 MHz, D_2O) spectrum of **3**.

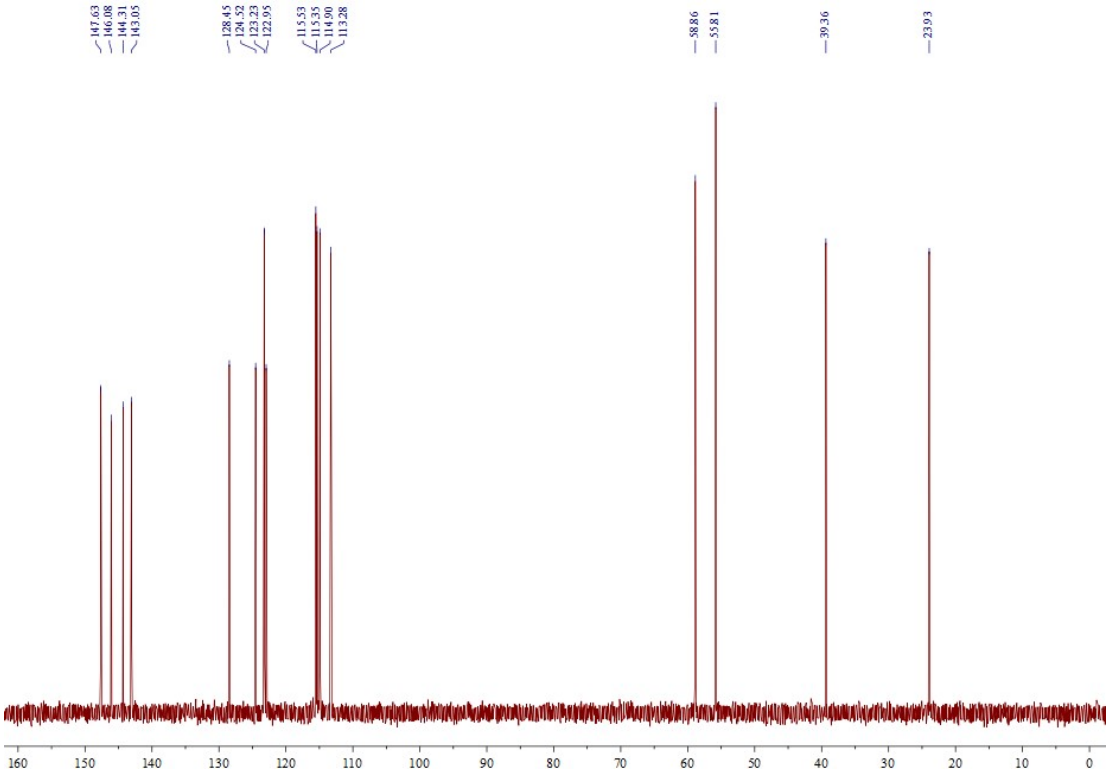

Figure S10: ^{13}C-NMR (125 MHz, D_2O) spectrum of **4**.

S.3: DPPH assay

To test the ability to neutralize DPPH free radicals, 0.1 mL of the tested sample was added to 1.0 mL of a methanol solution of DPPH$^{\bullet}$ (diluted to an absorbance of 0.7-1.0)[3]. The obtained mixture was incubated for 30 minutes in the dark, after which the absorbance was measured at 517 nm. Ascorbic acid was used as a positive control. The percent neutralization/reduction of DPPH$^{\bullet}$ was calculated according to the equation below, and the results are presented as IC_{50} values.

$$\%\ \mathrm{DPPH}^{\bullet} = \left(1 - \frac{A_t}{A_0}\right) \times 100$$

A_t - Absorbance of DPPH$^{\bullet}$ after 30 minutes; A_0 - Absorbance of DPPH$^{\bullet}$

S.4: ABTS assay

To test the antioxidant activity using this method, the radical cation species was generated by mixing equal volumes of 7 mM ABTS and 2.45 mM $K_2S_2O_8$ solutions and incubating at 4°C for 16 hours[3]. Different concentrations of the tested samples (0.1 mL) were added to 1 mL of the thus prepared ABTS$^{\bullet+}$ solution (diluted to an absorbance of 0.7-1.0) and the absorbance was measured after 7 minutes at 734 nm. Ascorbic acid was used as a positive control. The percent neutralization/reduction of ABTS$^{\bullet+}$ was calculated according to the equation below, and the results are presented as IC_{50} values.

$$\%\ \mathrm{ABTS}^{\bullet+} = \left(1 - \frac{A_t}{A_0}\right) \times 100$$

A_t - Absorbance of ABTS$^{\bullet+}$ after 7 minutes; A_0 - Absorbance of ABTS$^{\bullet+}$

S.5: FRAP assay

To monitor the reduction of Fe^{3+} ions by this method, the procedure by Benzie and Strain was used[3]. Stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tris(pyridin-2-yl)-*s*-triazine (TPTZ) in EtOH, and 20 mM $FeCl_3$ in 20 mM HCl. To prepare the working FRAP solution, acetate buffer, TPTZ and $FeCl_3$ solution were mixed in a volume ratio of 10:1:1 and incubated as such at 37°C before use. To 3 mL of FRAP reagent and 0.3 mL of water, an aliquot of the sample (0.1 mL) was added. All measurements were adjusted to 593 nm after 6 minutes. The obtained results are expressed as ascorbic acid equivalents (*AAE*).

S.6: ORAC assay

Oxygen radical absorption capacity was measured according to the modified procedure described in the work of Cao et al.[4]. The prepared mixture of 0.05 mL of fluorescein solution (0.42 µM), 0.1 mL of the tested sample (1 µM) and 1.8 mL of phosphate buffer (0.1 M, pH 7.3) was incubated at 37°C for 15 minutes. After the incubation, ROO$^{\bullet}$ formation was initiated by the addition of 0.05 mL of AAPH solution (640 mM). A 1 µM Trolox solution was used as a standard. All measurements were made in triplicate by measuring the fluorescence intensity for 60 minutes (λ_{ex} 485 nm, λ_{em} 520 nm). The results obtained by this method are expressed as equivalents of Trolox, and are calculated according to the formula:

$$\mathrm{ORAC}\left(\frac{\mu\mathrm{mol_T}}{\mu\mathrm{mol_S}}\right) = k \times \frac{S_S - S_B}{S_T - S_B}$$

k - Dilution factor; S - Areas under the curves; S - Sample; B - Blank; T - Trolox

$$S = \frac{1}{2} \times \left[(t_1 - t_0) \times (f_1 + f_0) + \ldots + (t_{i+1} - t_i) \times (f_{i+1} + f_i)\right]$$

t - time (min); f - Relative fluorescence intensity

S.7: Fe(II) chelating assay

The most important spectrophotometric method for monitoring the chelation of Fe^{2+} ions is the competitive method of complex formation with ferrozine in the presence of potential ligand[5]. To 0.05 mL of $FeCl_2$ solution (2 mM), 0.4 mL of the solution of the tested compound was added. The reaction was initiated by adding 0.2 mL of ferrozine solution (5 mM). The total volume to 4 mL was made up with EtOH, the solution was mixed and incubated at room temperature for 10 minutes. Na_2EDTA was used as a positive control. The absorbances of the solutions prepared in this way were measured at 562 nm, and the percentage of chelation was calculated according to the equation.

$$\% \text{ Chelation} = \left(1 - \frac{A_t}{A_0}\right) \times 100$$

A_t - Absorbance after 10 minutes; A_0 - Absorbance of the control

S.8: Inhibition of AChE

The potential AChE-inhibitory action of synthesized THIQs was determined by Ellman's method[6]. Galantamine hydrobromide was used as a positive control, while the final concentrations of the tested compounds in the reaction mixture were 0-50 µM. A mixture of 0.3 mL of phosphate buffer (100 mM, pH 8.0), 0.3 mL of sample and 0.3 mL of AChE (0.54 U/mL) was incubated for 15 minutes at 37°C. At the end of the incubation, 0.3 mL of acetylcholine iodide (15 mM) and 1.5 mL of 5,5-dithiobis-(2-nitrobenzoic acid) solution (3 mM) were added. For the resulting mixtures, the absorbance was measured at 405 nm after 5 minutes. The percentage of inhibition was determined in relation to the control according to the formula:

$$\% \text{ Inhibition} = \left(1 - \frac{A_t}{A_0}\right) \times 100$$

A_t - Absorbance after 5 minutes; A_0 - Absorbance of the control

Based on the results, a linear equation was created indicating the correlation of the common logarithm of the concentration of the tested sample and the percentage of AChE inhibition, from which the IC_{50} values were extrapolated.

S.9: Docking studies

Molecular docking and visualization were performed using Autodock Vina[7] and PyMOL[8]. The geometry optimized structure of THIQs was exported as a pdb file. For docking study, the THIQs non-polar hydrogens were merged and rotatable bonds were defined. The crystal structure of acetylcholinesterase (AChE) from *Electrophorus electricus* (PDB code 1C2B) was obtained from the Protein Data Bank[9]. Water molecules were removed, and the atomic coordinates were stored in a separate file and used as input for AutoDock Tools. Thus, polar hydrogens were added to protein structure and Kollman united atom partial charges were assigned. The binding site were defined using grid size coordinates of 60×60×72 and grid center coordinates of x=21.5911, y=87.752, z=23.591 with a grid space of 0.375 Å[10]. During the docking process torsional bonds of ligand were set free. The protein-ligand complex was visualized and analyzed using PyMOL software.

S.10: Antibacterial activity

Agar well method was used to evaluate the antibacterial potential of the synthetic substances[11]. The referent strains that were tested in the study included *Staphylococcus aureus* (ATCC 6538), methicillin resistant *Staphylococcus aureus* (ATCC 33591), *Pseudomonas aeruginosa* (ATCC 10145) and *Escherichia coli* (ATCC 14169). A bacterial inoculum of 0.5 McFarland density was applied to Mueller Hinton agar plates. Wells were made in agar (diameter of 8 mm) and 100 µL of the tested compounds was added to each well. Ampicillin was used as a positive standard. Each

experiment was done in triplicate. After overnight incubation at 37°C zones of inhibition were measured and results were expressed in the form of zones of inhibition measured in mm.

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