**Supplementary file**

## 2. Experimental

### 2.1. Chemical synthesis

All melting points were uncorrected and determined on an electrothermal melting point apparatus (Stuart Scientific, UK). Elemental microanalyses (C, H, N) were performed on a model 2400 CHNSO analyzer (Perkin Elmer, USA). All compounds were within ±0.5 % of the theoretical values. Infrared spectra were recorded on a FT-IR 6100, Fourier transform, infrared spectrometer at cm-1 scale using KBr disc technique (JASCO, Japan). $^{1}$H NMR and $^{13}$C NMR spectra were determined by using a JEOL AS-500 NMR spectrometer (JEOL, Japan), chemical shifts are expressed in $\delta$ (ppm) downfield from TMS as an internal standard. The mass spectra were measured with a GC MS-Qp1000EX Shimadzu (Shimadzu, Japan). Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Germany) using chloroform/petroleum ether 40-60 (5:1, *V/V*) and the spots were detected by exposure to UV lamp at λ 254 nanometer for few seconds and by iodine vapour.

#### 2.2.1.1. Acetylcholinesterase Inhibition Screening Protocol

Acetylcholinesterase (AChE) inhibitory activity was measured using quantitative colorimetric assay according to the method developed by Ellman *et al.* [17]. The results were given as $IC_{50}$ (μg/ml) and the percentage of inhibition was determined by the comparison of reaction rates of samples relative to the blank sample (methanol in phosphate buffers, pH 8) using the formula: Percentage of inhibition (%) = [(Ac −As) / Ac] ×100 Where Ac and AS are the absorbance of the reference and sample obtained from the UV–visible spectrophotometer, respectively.

### 2.2.2. *In vivo* studies

#### 2.2.2.1. Animal Care

Wistar albino rats weighing between 180–200 g were housed in the animal house of National Research Centre. The rats were kept in cages and maintained under standard conditions (12:12 h light/dark cycle, controlled room temperature (23 ± 2°C), stress-free, *ad libitum* water, standard diets, and sanitary conditions). Before commencing the experiment, the rats were allowed to acclimatize for a period of 1 week to reduce stress.

#### 2.2.2.3. Toxicity study

Selected 8 Wistar rats of uniform weight were taken and divided into two groups of 4 rats each for each concentration. The synthesized compound **4c** was given orally to rats in doses 50 and 100 mg /kg b.wt Desoukey *et al.* [19]. The control group (4 rats) received the same volumes of distilled water. The percentage mortality for 4c was recorded 24 h later. Observation of rats continued for one month, for mortality, and any changes in the skin, respiratory, and behaviour pattern. Particular observation for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma was done as described by Desoukey *et al.* [19].

**2.2.2.5. Biochemical Analyses**

**2.2.2.5.1. *In vivo* studies**

After one month, rats were anaesthetized with diethyl ether, blood was drawn via sublingual vein rupture, and rats were decapitated. Blood samples were centrifuged at 3000 rpm, and the clear serum that was separated was frozen at -80 °C for biochemical analysis. The whole brain was rapidly dissected on an ice-cooled glass plate, thoroughly washed with isotonic saline, dried, and divided into two portions. The first portion was homogenised to give 10% (w/v) homogenate in an ice-cold medium. The second portion of the brain will be used for histopathological determination. Hepatic function was evidenced by aminotransferase (AST), alanine aminotransferase (ALT), which was determined according to [21], and alkaline phosphatase (ALP), which was determined according to the method of Belfield and Goldberg [22]. Renal function was evidenced by urea and creatinine [23, 24]. Hematological analysis was performed using an automatic hematological analyzer (Cell Dyn, Abbott). Hb, RBCs, HCT-MCV, MCV, MCH, MCHC, RDW-CV, platelet count, MPV, and WBCs were the haematological parameters that were assessed [25].

**2.2.2.5.3. Behaviour Studies**

The neurocognitive function of rats was estimated by T-maze test (constructed in the NRC, Egypt) according to Deacon and Rawlins [27]. Before performing this experiment, the animals were left without food for 24 h, with only water to drink. The groups were subjected to the rewarded T-maze test which was done thrice: at zero time before starting oral induction with $AlCl_3$, after AD-induction period by 24 h, and 9 days (5 rats from the AD group were anaesthetized and sacrificed at this stage), and 24 h after the last oral treatment with the tested materials and behavioural observations will be recorded. At the end of the experimental period, rats were anaesthetized for blood collection. Then, they were sacrificed for brain homogenates to

the estimate the molecular, inflammatory and oxidative stress markers as well as immuno and histopathological studies which are in progress. The cognitive ability and impairment of spatial memory of the rats were evaluated after chronic administration of AlCl3 (two months) at the end of the treatment period [28]. Motor ability was assessed using a beam balance test [29].

**2.2.2.6.2. Brain tissue sampling preparation**

At the end of the experiment, the rats were fasted overnight, subjected to anesthesia and scarified. The whole brain of each rat was rapidly dissected, washed with isotonic saline and dried on a filter paper. The brain was weighed and homogenized in ice-cold medium containing 50 μM Tris/HCl and 300 μM sucrose at pH 7.4 to give a 10% (w/v) homogenate. This homogenate was centrifuged at 1400 x g for 10 min at 4 $^0$C. The supernatant was kept at -80 $^0$C and used to test for oxidative stress biomarkers such as MDA and GSH. All animal groups were subjected to a determination of non-enzymatic glutathione (GSH) reduction according to the method of Beutler *et al.* [30], where the method was based on the reduction of 5,50-dithiobis(2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration, and its absorbance can be measured at 405 nm. For malondialdehyde (MDA), thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in an acidic medium at a temperature of 95 $^0$C for 30 min to form a thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm [31].

**2.1. Molecular docking studies**

The crystal structure of acetylcholinesterase (PDB:1EVE) was retrieved from Protein Data Bank (http://www.rcsb.org). Protein is prepared with Molecular Operating Environment (MOE) software; the water was removed, and the hydrogen atoms were added using MOE. A ligand structure was built with MOE and energy minimized usingtheMMFF94x force field until a RMSD gradient of 0.05 kcal mol_1Å_1 was reached. Compound **4c** was docked within the active site of the acetylcholinesterase protein. To validate the docking protocol, the native ligand was re-docked within the active site. In the present docking protocol, the placement criterion was chosen to be "Alpha Triangle." Rescoring 1 was set to be London G and retain 10 poses. In this study, it was preferred to refine with a force field, and rescoring 2 was chosen to be London G.

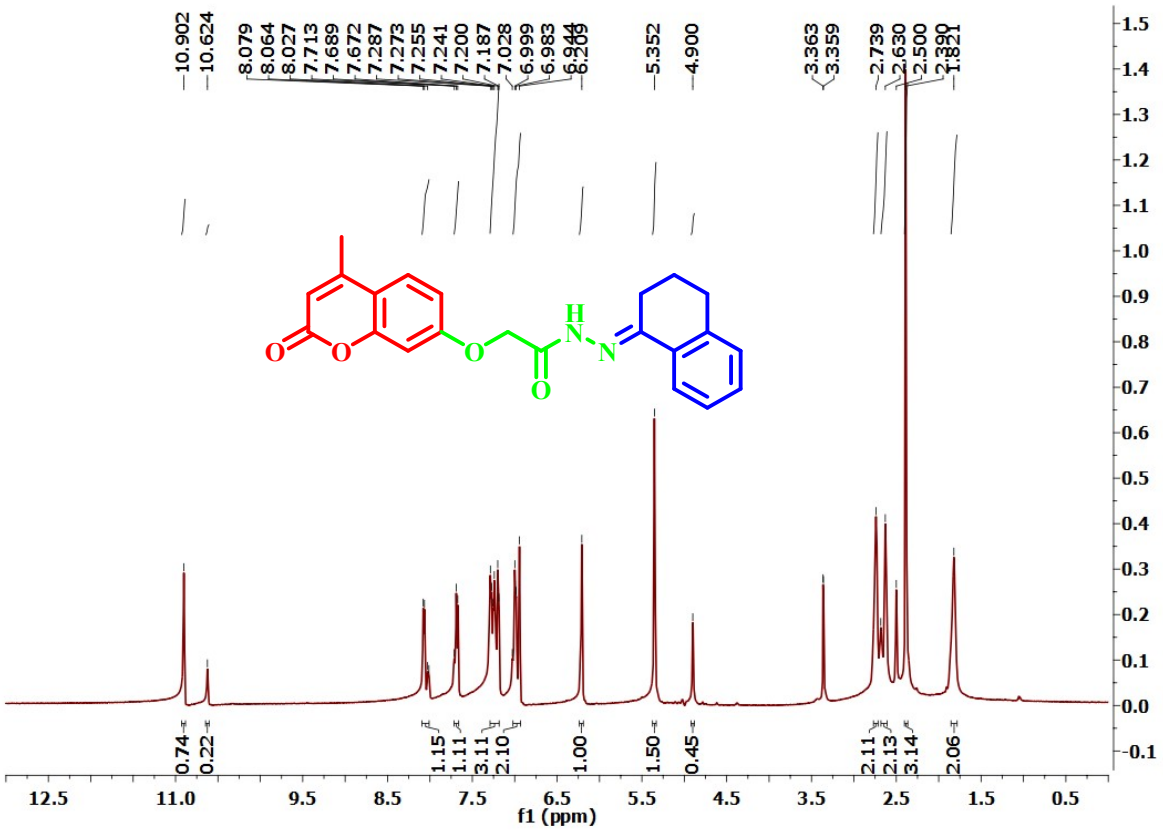


**Fig. 1.**[1]H-NMR spectrum of compound **4a**

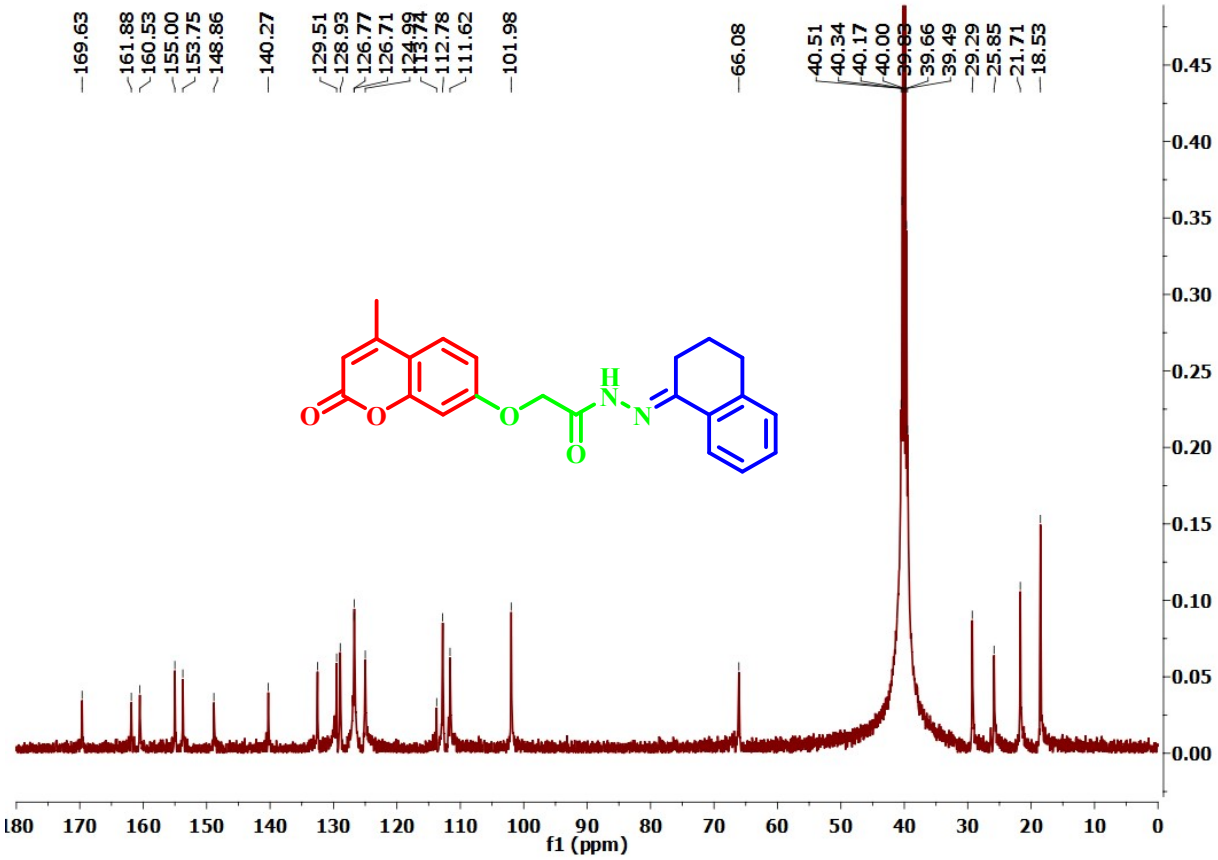


**Fig. 2.** $^{13}$C-NMR spectrum of compound **4a**

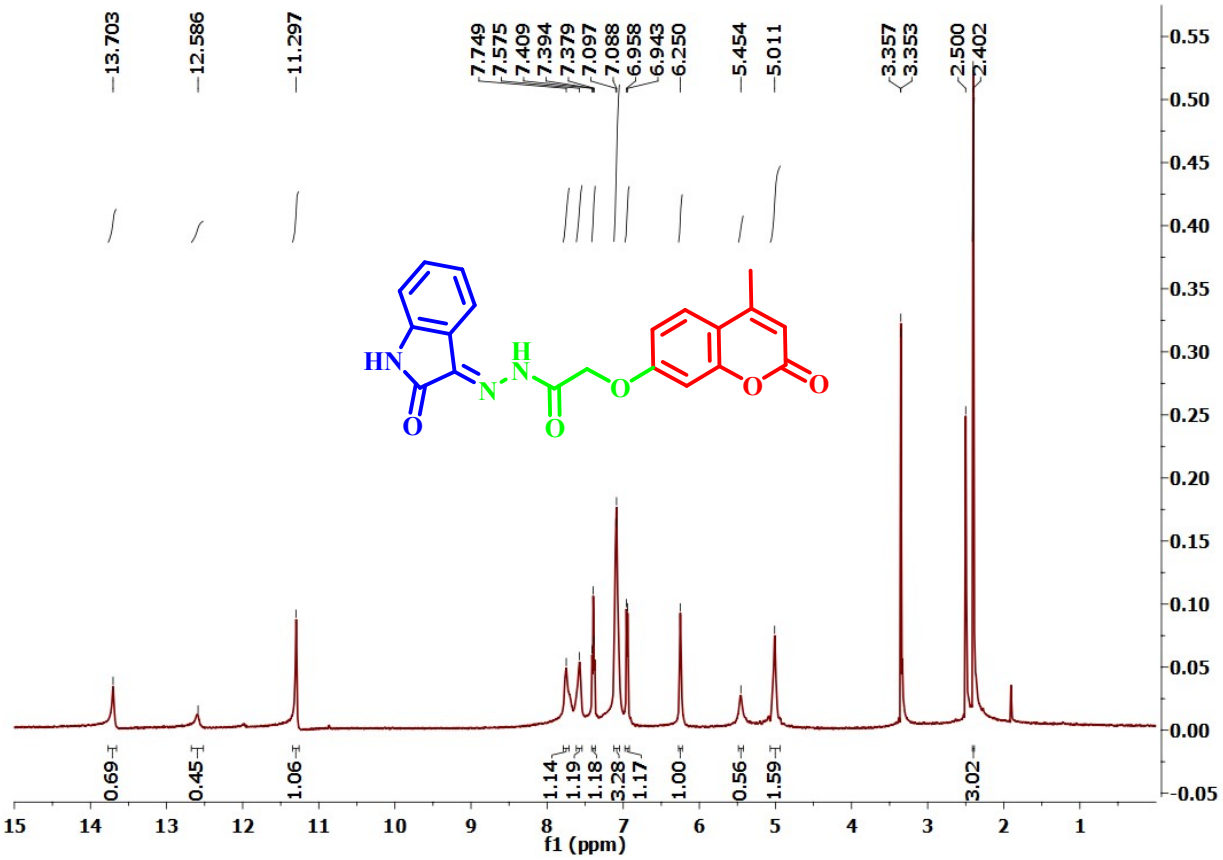


**Fig. 3.**$^{1}$H-NMR spectrum of compound **4b**

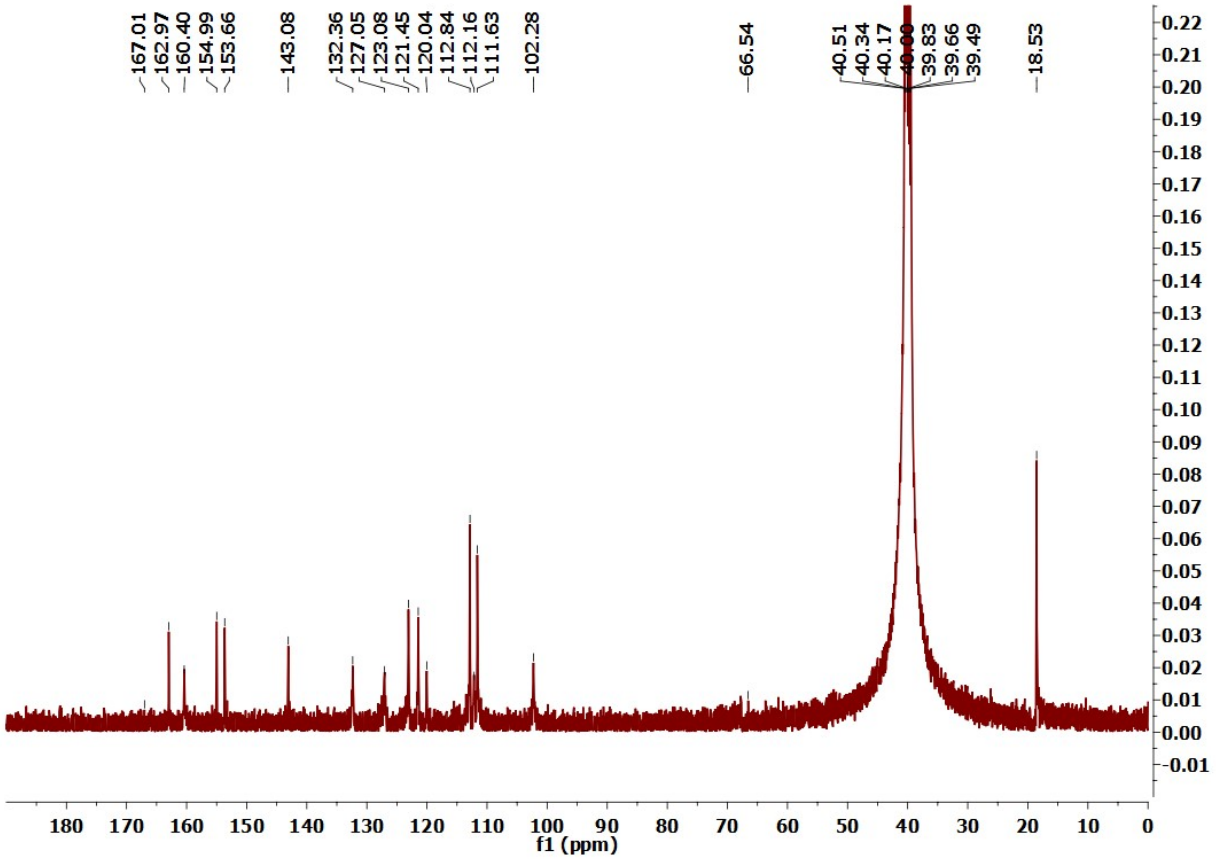


**Fig. 4.** $^{13}$C-NMR spectrum of compound **4b**

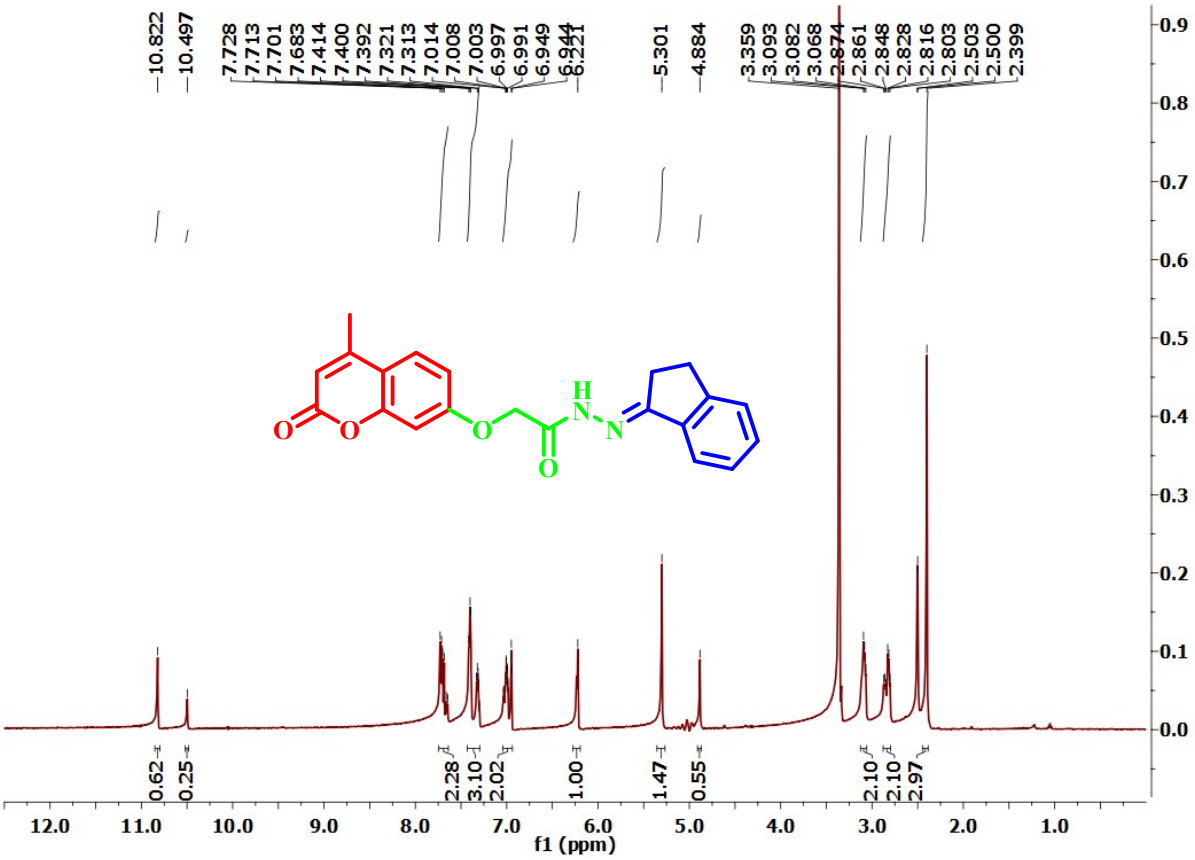


**Fig. 5.**[1]H-NMR spectrum of compound **4c**

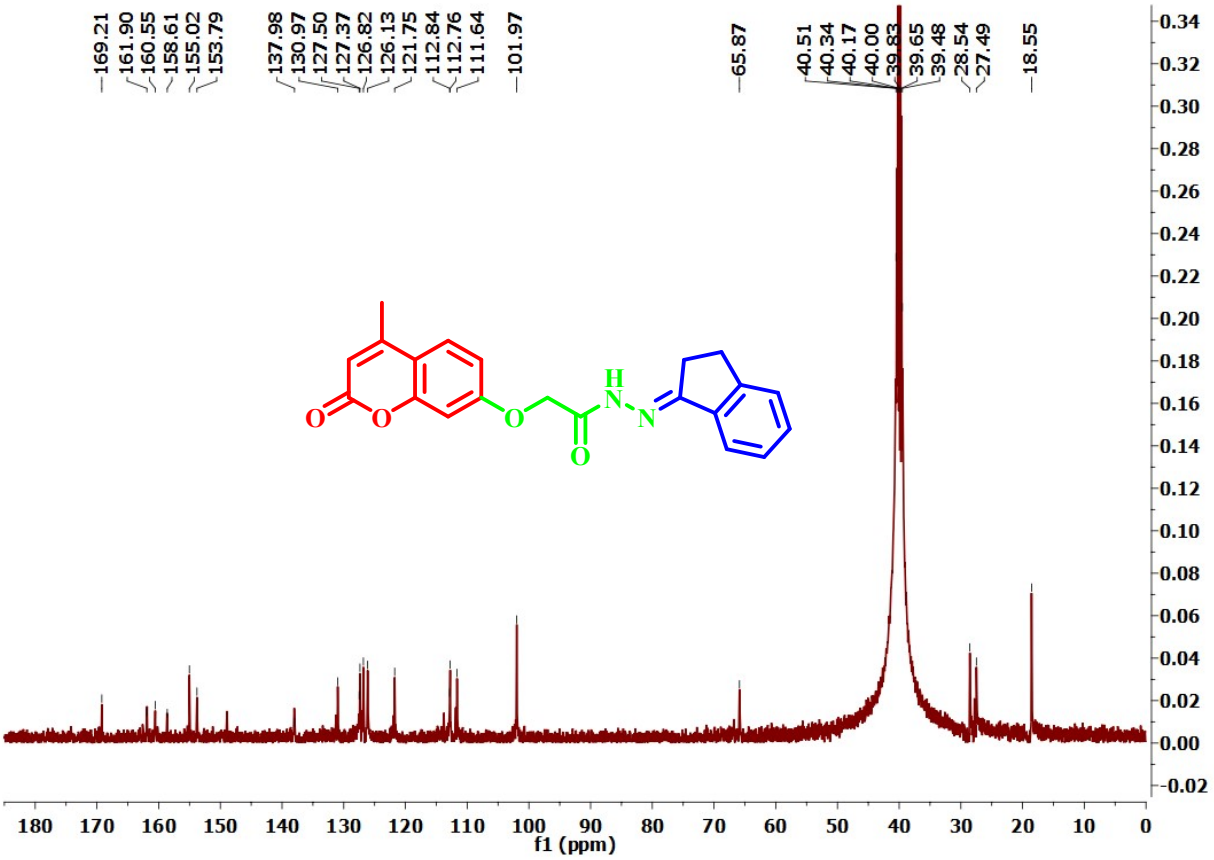


**Fig. 6.**$^{13}$C-NMR spectrum of compound **4c**

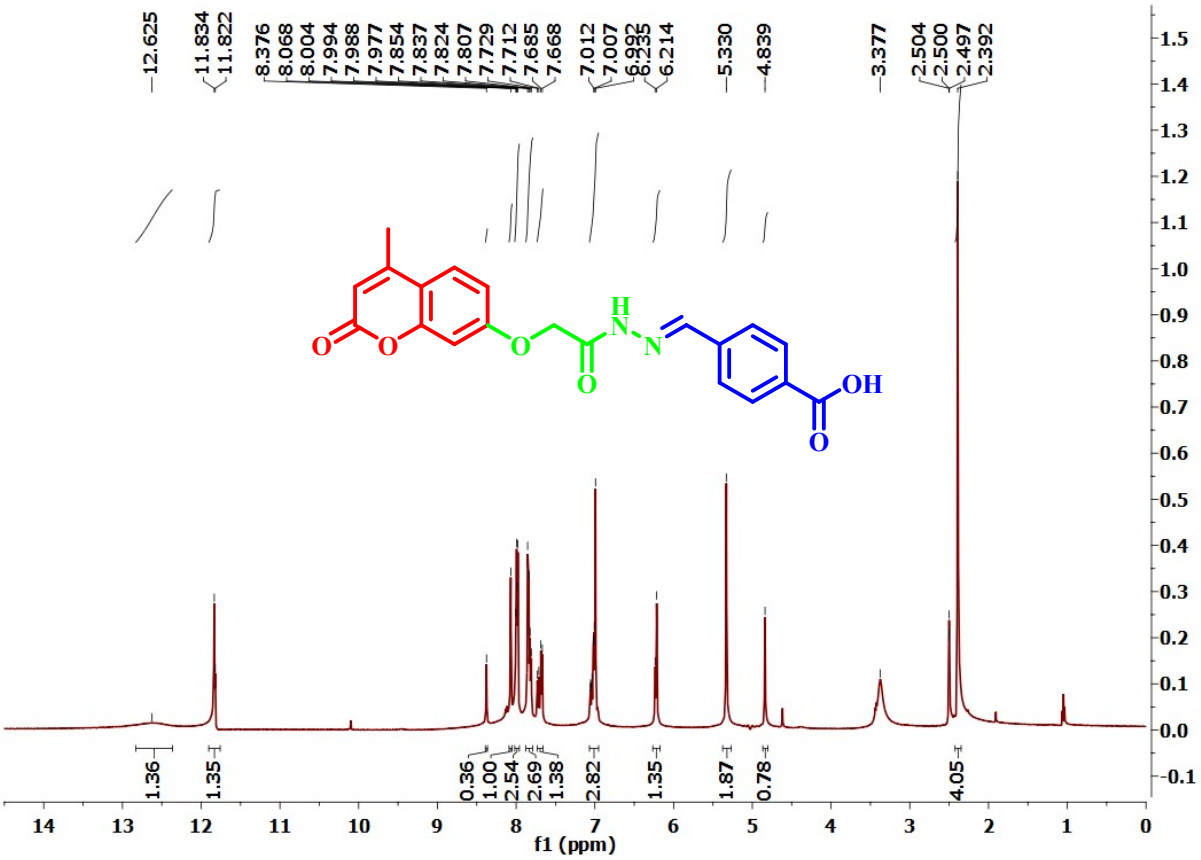


**Fig. 7.**[1]H-NMR spectrum of compound **4d**

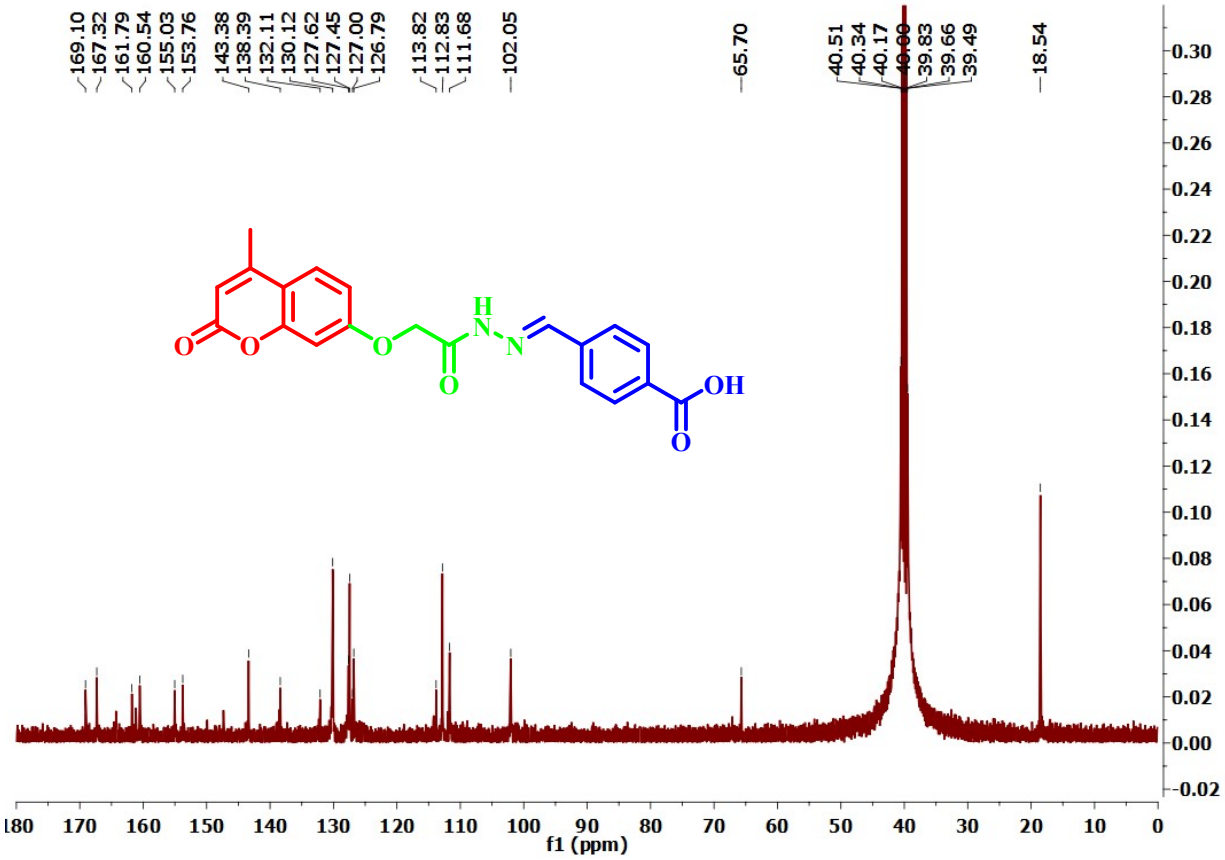


**Fig. 8.** $^{13}$C-NMR spectrum of compound **4d**