Synthesis, acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) Activities and Molecular Docking Studies of Novel Compound based on Combination of Flurbiprofen and Isoniazide

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Experimental section

All of the chemicals and reagents were purchased from Sigma Chemical Co. USA and used without further purification. The solvents were dried by usual techniques. The TLC plates used were silica gel GF-254 (E. Merck, Germany) with 1:1 ethyl acetate: petroleum ether as the mobile phase. Melting point was determined by using Gallenkamp melting point apparatus and reported uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV400 and Bruker DPX 200 spectrometers, respectively. The chemical shifts were reported in parts per million on the δ scale with reference to tetramethylsilane. The J values were reported in Hz. ESI-MS spectra were recorded on Waters LCT Premier Open Access System.

Synthesis of flurbiprofen chloride (A)

Flurbiprofen (1.5000 g, 6.14 mmol) was dissolved in dry dichloromethane (30 mL). To this dry DMF (0.03 mL) was added as catalyst. This was followed by drop wise addition of oxalyl chloride (~3 mL) as the chlorinating agent; during the addition temperature was maintained at 0-5ºC. The resulting mixture was stirred for 12 h at about 25ºC. After this the solvent was evaporated on a rotary evaporator. An oily product was obtained. The product produced single spot in the TLC. Yield: 93.1%. The elemental analysis of the purified product gave CHN analysis conforming to $C_{15}H_{12}FOCl$ composition within 0.3% experimental error. ^{1}HNMR $CDCl_3$(δ); 7.48 (m, 2H, H-12, H-13), 7.41 (m, 1H, H-14), 7.32 (m, 2H, H-10, H-11), 7.22 (m, 1H, H7), 6.9 (m, 2H, H-5, H-6), 3.8 (q, J=7.0 Hz, 1H, H-3), 1.45 (d, J=6.9 Hz, 3H, H-2); ^{13}C-NMR: 173.6 (C-1), 140.3 (C-8), 139.1 (C-10), 130.0 (C-7), 129.0 (C-9), 128.7 (C-5), 118.7 (C-6), 54.5 (C-3), 16.4 (C-2).

Synthesis Novel Compound of flubiprofen and isoniazid, N'-(2-(2-fluoro-[1, 1'-biphenyl]-4-yl) propanoyl) isonicotinohydrazide (1)

To a stirred solution of isonicotinohydrazide (109.7 mg, 0.80 mmol) in dry dichloromethane (15 mL) triethyl amine (0.3 mL) was added under inert atmosphere. This was followed by addition of flurbiprofen chloride (209.6 mg, 0.80 mmol) and 4-dimethylaminopyridine (12 mg, 0.098 mmol) in dry dichloromethane (15 mL). The reaction mixture was stirred overnight. After this the reaction was found to be complete as evidenced by no further change in TLC pattern (R_f = 0.4) using ethyl acetate and petroleum ether solvent (1:1). The solvent was evaporated by rotary evaporator. A white powder was isolated by filtration. The product was purified by column chromatography (Silica gel, 1:1 ethyl acetate: petroleum ether). The product was dried under reduced pressure. Yield: 211 mg (72 %).

λ_{max} 270 nm; IR (KBr) ν cm-1: 3468 (amide), 1595 (–C=O), 1323 (–CN), 1143 (–CF), 836 (–Ar)

^{1}H-NMR $CDCl_3$ (δ); 8.8 (m, 2H, H-20, H-19), 8.2 (br s, 2H, –CONH), 7.52 (m, 2H, H-3, H-5), 7.51 (m, 2H, H-2, H-6), 7.48 (m, 1H, H-12), 7.41 (m, 1H, H-1), 7.3 (m, 2H, H-18, H21), 7.20 (d, J=2.20 Hz, 1H, H-9), 7.15 (m, 1H, H-11), 3.8 (q, J=6.9 Hz, 1H, H-13), 1.55 (d, J=6.8 Hz, 3H, H-14, H14A, H14B); ^{13}C-NMR (δ): 172.2 (C-15), 171.2 (C-16), 169.0 (C-8), 150.1 (C-20), 143.1 (C-17), 136.8 (C-10), 135.1 (C-4), 129.2 (C-2), 128.8 (C-7), 127.8 (C-12), 127.6 (C-1), 127.2 (C-5), 123.4 (C-11), 123.1 (C-18), 117.1 (C-9), 47.5 (C-13), 18.5 (C-14); ESI-MS: 364.1456 [M+H$^+$], 365.1446; $C_{21}H_{18}FN_3O_2$.

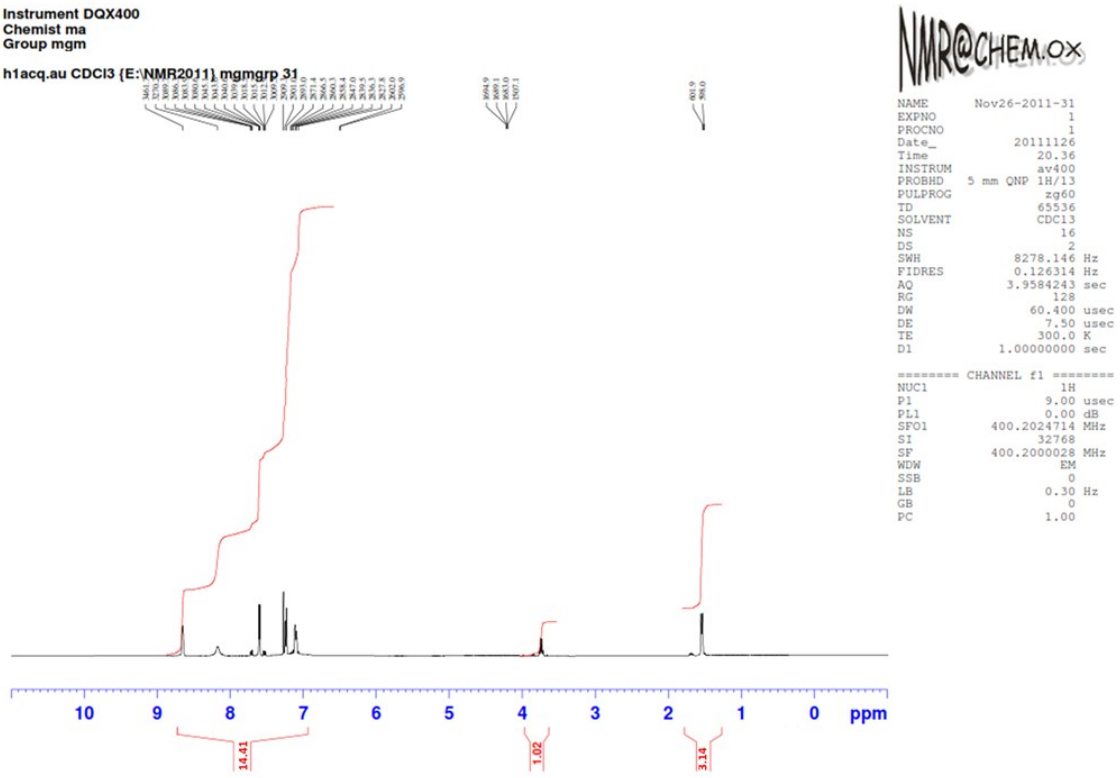

Figure S1: ^{1}H NMR of Novel Compound of flubiprofen and isoniazid, N'-(2-(2-fluoro-[1, 1'-biphenyl]-4-yl) propanoyl) isonicotinohydrazide (1)

Biological Activities

Anti-inflammatory activities

The anti-inflammatory activity of the drugs under investigation was determined by inhibition of kaolin paw edema. The edema was induced in male Wistar rats (95-110 g) in groups of five by a reported method [1, 2]. The drugs were administered orally in 5% edible oil (Rafhan Maize Products Company Ltd., Faisalabad) in distilled water (0.2 mL/ 100 g body weight) 1 h before the kaolin. The rats were dosed on a weight of drug (mg) per body weight (kg) of animal basis. Edema was evaluated

4 h after the subplantar administration of kaolin in 0.9% w/v sodium chloride solution. Inhibition of oedema was calculated by comparing the swelling obtained in treated animals with the controls, and was expressed as % inhibition. Statistical significance was determined by use of Student t-test ($P<0.05$).

All the procedures involving animals and biological materials were in accordance with the current revision of the Helsinki Declaration and International Principles for the Biomedical Research Involving Animal (CIOMS/OMS, 1985). The study was approved by the Ethics Committee of the university.

Enzyme inhibition studies

All the assays were carried out in triplicate. The percentage inhibition was computed using the formula:

$$Inhibition\ (\%) = \frac{(Absorbance\ of\ control - Absorption\ of\ test\ solution)}{Absorbance\ of\ Control} \times 100$$

IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

AChE inhibition

The AChE assay was performed according to Ellman method [3, 4] using 10 µL per well of the test compound (0.5 mM) and acetylchline iodide as the substrate. Physostigmine (Eserine) was used as a positive control. Absorbance was recorded at 405 nm.

BuChE inhibition

The BuChE inhibition activity was performed according to Ellman method [4] by using 10 µL per well of test compound (0.5 mM) and butyrylthiocholine bromide as the substrate. Here again physostigmine (Eserine) was used as a positive control. Absorbance was recorded at 405 nm.

Determination of LD_{50}

The LD_{50} value of the synthesized novel drug was determined by use of albino Wistar male rats, 175-230 g. Animals were kept at 23±0.5°C and constant humidity. Conventional laboratory diet and water were freely available. The drug was administered orally in 0.15% agar suspension to four groups of ten rats each. After treatment, the animals were monitored every hour for 12 h and then every day for 14 days.

Docking analysis

The Compound (**1**) was subjected to computational analysis for evaluation of druglikeness, bioavailability and lipophilicity using Spartan. [5] The validity of the calculations was verified by comparing the calculated properties with the experimental values of the parent drugs. The results confirm the molecule complies with Lipinski's rule of five for druglikeness as the logP, molecular

weight, molar refractivity and number of atoms were between -0.4-5.6, 160-480, 40-130 and 20-70, respectively. The number of H-bond donors and acceptors were <5 and <10 respectively.

The docking analysis was calculated by binding the compound (**1**) with BuChE which served as the target proteins which have the following PDB ID- 1P0I. [6] Autodock vina was used to dock the compound (**1**) to the target protein. [7] Compound (**1**) showed improved binding affinity with *BuChE* compared to flurbiprofen.

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