Supplementary file

Development of pyrazolo[1,5-*a*]pyrimidine derivatives: Synthesis, anticancer activity and docking study

1. Experimental

1.1 Chemistry

Melting points were measured in open capillary tubes using Electro thermal apparatus and are uncorrected. Elemental microanalyses were carried out at the regional centre for Mycology and Biotechnology, AL-Azhar University. The infra-red (IR) spectra were recorded using potassium bromide disc technique on Schimadzu 435 IR Spectrophotometer at Microanalytical unit, Cairo University. ^{1}H NMR and ^{13}C NMR spectra were performed on a Varian Mercury VX-300 NMR spectrophotometer 300 MHz or on Agilent Technologies 400 MHz NMR spectrophotometer at the Armed Forces Laboratories. DMSO-d_6 was used as a solvent, and the chemical shifts were measured in *ppm*, relative to TMS as an internal standard. As for the proton magnetic resonance, D_2O was carried out for NH and OH exchangeable protons. Mass spectra were recorded on a DI-50 unit of Shimadzu GC/ MS-QP 2010 plus Spectrometer (Japan) or on single quadrpole mass Spectrometer ISQ LT (Thermo scientific) and carried out at the regional center for Mycology and Biotechnology, AL-Azhar University. All reactions were monitored by TLC using pre-coated Aluminum sheet silica gel Merck 60 F 254 and were visualized by UV lamp. Chemical naming and calculated microanalysis of new compounds were performed by ChemDraw Program 12.0 software [Chemical Structure Drawing Standard; Cambridge Soft Corporation, USA (2010)].

1.2 Biology

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan. [98] All the following procedures were done in a sterile area using a Laminar air flow cabinet of class II biosafety (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in DMEM medium for PC-3, and RPMI1640 in case of A-549, MCF-7, HCT -116 and HePG-2. The media are supplemented with 1% antibiotic antimycotic mixture (10,000 U ml^{-1} Potassium Penicillin, 10,000 μg ml^{-1} Streptomycin Sulfate and 25 μg ml^{-1} Amphotericin B), 1% L-glutamine and 10% fetal bovine serum (complete growth medium) and kept at 4 °C under 5% CO_2. Cells were batch cultured for 10 days, then seeded at concentration of 10x103 cells/well in fresh complete growth medium in 96-well Microtiter plastic plates at 37 °C for 24 h under 5% CO_2 and a humid atmosphere using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with a serial dilutions of each sample. After 48 h of incubation, the medium was aspirated, 40 μl MTT salt (2.5 μg ml^{-1}) were added to each well and incubated for a further four hours at 37 °C under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 μl of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37 °C. [1] The absorbance was then measured using a microplate multi-well reader ELISA reader) (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration in the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula: (average absorbance of extract/ average absorbance of negative control)-1) x 100. A probit analysis was carried for IC_{50} determination using SPSS 11 program.

1.3 Docking

All the molecular modeling calculations and docking simulation studies were performed using Molecular Operating Environment (MOE®) 2014. All the interaction energies and different calculations were automatically calculated. Optimization of Target compounds 2(a-c) was constructed as a 3D model using the builder interface of the MOE program. After checking its structure and the formal charges on atoms by 2D depiction, the following steps were carried out: the target compound 2(a-c) were subjected to a conformational search. All conformers were subjected to energy minimization; all the minimizations were performed with MOE until a RMSD gradient of 0.01 Kcal mol-1 and RMS distance of 0.1 Å with MMFF94X force-field, and the partial charges were automatically calculated. The obtained database was then saved as MDB file to be used in the docking calculations.

The three dimensional X-ray structures of CDK2 (PDB code: 2A4L) was obtained from the Protein Data Bank through the internet. The enzymes were prepared for docking studies by removing the ligand molecules from the active sites. Hydrogen atoms were added to the system with their standard geometry. Atoms'

connection and type were checked for any errors with automatic correction. Selection of the receptor and its atoms' potential were fixed. MOE Alpha Site Finder was used for the active sites search in the enzymes' structures using all default items. Dummy atoms were created from the obtained alpha spheres. Re-docking of co-crystalline ligands to the receptors' active sites to insure the docking method was efficient. Docking results were visualized using Biovia discovery-studio 2020 visualizer, which provided 3D and 2D estimated interactions,

References:

1. B.S. El-Menshawi, W. Fayad, K. Mahmoud, S.M. El-Hallouty, M. El-Manawaty, M.H. Olofsson and S. Linder. Screening of natural products for therapeutic activity against solid tumors. *Indian J. Exp.Biol.*, vol. 48, no. 3, pp. 258-264, 2012.