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Name of Topic: Metal catalyzed Synthesis of Heterocyclic Derivatives and Their Biological Evaluation Keywords: Pyrazoline, Suzuki coupling, Anticancer activity, MD simulation, ADME Assay, DNA binding, Antioxidant studies.

Findings

The analogs 3m and 3n were synthesized by Suzuki coupling reaction in the presence of $Pd(PPh_3)_4$ as catalyst. The structural elucidation of N-formyl pyrazoline derivatives was carried out by various spectroscopic techniques such as ^1H, ^{13}C NMR, FT-IR, UV-visible spectroscopy, mass spectrometry and elemental analysis. Anticancer activity of the derivatives (3a-3l) was evaluated against human lung cancer (A549), fibrosarcoma cell lines (HT1080) and human primary normal lung cells (HFL-1) by MTT assay. In vitro cytotoxicity of analogs 3m and 3n against human breast cancer cell line (MCF-7) and human normal cell (HepG2). The results showed that potent analogs 3b and 3d exhibited promising anticancer activity against A549 (IC_{50} = 12.47±1.08 and 14.46±2.76 μM) and HT1080 (IC_{50} = 11.40±0.66 and 23.74±13.30 μM) but low toxicity against the HFL-1 (IC_{50} = 116.47±43.38 and 152.36±22.18 μM). The anticancer activity of potent derivatives (3b and 3d) against A549 cancer cell line was further confirmed by flow cytometry based approach. DNA binding interactions of the derivatives 3b and 3d have been carried out with calf thymus DNA (Ct-DNA) using absorption, fluorescence and viscosity measurements, circular dichroism and cyclic voltammetry. Antioxidant potential of N-formyl pyrazoline derivatives (3a-3n) has also been estimated through DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical and H2O2. The results revealed that all the derivatives exhibited significant antioxidant activity. In silico molecular modelling and ADMET properties of pyrazoline derivatives were also studied using PyRx software against HuTopII receptor with PDB ID: 1ZXM to explore their best hits. Molecular dynamics (MD) simulations of 3b and 3d were also carried out with HuTopII for structure-function correlation in a protein. The compounds 3p-3r were synthesized via Suzuki coupling reaction in the presence of $Pd(PPh_3)_4$ [tetrakis-(triphenylphosphine)palladium(0)] as a catalyst with good yield. All synthesized derivatives were fully characterized using several techniques such as 1H, 13C NMR, FT-IR spectroscopy, and mass spectral analysis. The molecular structure of compounds 3g-3i was validated by X-ray single crystallography. Anticancer activity of derivatives (3a-3o) was investigated by MTT assay against the human lung cancer cell (A549), human cervical cancer cell (HeLa) and human primary normal lung cells (HFL-1). In vitro cytotoxicity of 3p-3r derivatives against human breast cancer cell line (MCF-7) and human normal cell (HepG2). The results of anticancer activity showed that two potent analogs 3a and 3h exhibit excellent activity against A549 with IC_{50} = 13.49±0.17 and 22.54±0.25μM and HeLa cells with IC_{50} = 17.52±0.09 and 24.14±0.86 μM; and low toxicity against the HFL-1 with IC_{50} = 114.50±0.01 and 173.20±10μM, respectively. The flow cytometry was further used to confirm the anticancer activity of potent derivatives against the A549 cancer cell line. DNA binding interaction of anticancer agents 3a and 3h with Ct-DNA has been carried out by using absorption, fluorescence, circular dichroism, cyclic

voltammetry, and time-resolved fluorescence methods which showed noncovalent intercalation binding mode of interaction. derivatives was also performed using Autodock vina software 4.0 version of PyRx virtual screening tool against DNA hexamer with PDB ID: 1Z3F and ADMET properties to explore their best hits. three pyrazole analogs (4, 5a, 5b), pyrazole based chalcones (6a-6d), (8a-8h) and N-formyl/acetyl 1,3,5-trisubstituted pyrazole analogs (7a-7d), (9a-9d) were designed and synthesized. FT-IR, 1H, 13C NMR spectroscopy and mass spectrometry techniques were used to elucidate the structures of the analogs. Single X-ray crystallography method was used to identify the molecular structure of derivatives 4 and 5a. The synthesized analogs were screened by MTT assay against two cancer cell lines such as human lung cancer cell line (A549) and cervical cancer cell line (HeLa). Among all compounds, analog 9d demonstrates significant anticancer activity against HeLa (IC_{50} = 23.6μM) and A549 (IC50 = 37.59 μM). Anticancer activity of the derivatives was performed against human cancer cell (MCF-7) and human hepatoma cell line (HepG2) cell line by MTT assay. The results of anticancer activity showed that potent analogs 2b, 3b and 3e exhibited promising activity against MCF-7 cancer cell line with IC50 values of 1.25±0.3, 5.0±0.17 and 2.5±0.17 μM respectively but low toxicity against the HepG2. By using a flow cytometry-based technique, the anticancer effectiveness of potent compounds against MCF-7 cell line was further validated. Among all pyrazoline derivatives, analog 7d demonstrates potential anticancer activity against A549 cell line IC_{50} = 32.43 nM and IC_{50} = 79.47nM, against HEK293T cell line and compound 7a and 7b against HEK293T cell line with IC_{50} values of 15.39 and 21.20nM respectively.