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Structural and molecular docking studies of biologically active mercaptopyrimidine Schiff bases

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ABSTRACT

Novel Schiff bases derived from the treatment of mercapto-diamino pyrimidine with two different aldehydes are characterized using elemental analysis, single crystal X-ray diffraction and ^{1}H NMR spectroscopy. The pharmacological action of the synthesized compounds *viz*., antimicrobial, anticancer and antitubercular activities is studied. The Schiff bases show a very good activity against various test pathogens. DNA and β-CD binding interactions of the compounds are studied using UV–Visible absorption and fluorescence spectral measurements. The binding constants of the compounds towards β-CD are in the order of 10^3 to 10^4. Molecular docking is done using MOE program on the 3D structure of the enzymes, *viz*., human thymidylate synthase complexed with dump and raltitrex, candida albicans N-myristoyltransferasepeptidic inhibitor, catalytic domain of protein kinase pKnb from mycobacterium tuberculosis in complex with mitoxantrone, pare, topoisomerase atpase inhibitor, *E. coli* and lactobacillus casdihydrofolatereductase. The MIC/IC_{50} values of the Schiff bases are compared with the glide scores from the molecular docking studies. The number of hydrogen bonding interactions between the Schiff bases and amino acid residues are also reported.

1. Introduction

Schiff bases exhibit useful biological properties such as antiinflammatory, analgesic, antimicrobial, anticonvulsant, antitubercular, anticancer, antioxidant, anthelmintic, antiglycation and antidepressant activities. Schiff bases are also used as catalysts, pigments and dyes, intermediates in organic synthesis, polymer stabilizers and corrosion inhibitors. Pyrimidine derivatives and heterocyclic annulated pyrimidines continue to attract great interest due to their wide variety of interesting biological activities such as anticancer [1,2], antiviral [3], antitumor [4,5], anti-inflammatory [6,7] and antimicrobial [8,9]. Schiff bases attract much interest both from a synthetic and biological point of view [10,11]. Most of the reports in the literature reveal that Schiff bases derived from various heterocyclic compounds possess good cytotoxic [12,13], anticonvulsant, antiproliferative [14,15], antimicrobial [16,17], anticancer [18] and antifungal activities. They also show activity against gonadotropin releasing hormone receptors as well as herbicidal activity targeting acetohydroxy acid synthase, which catalyze the first common stepin branched-chain amino acid biosynthesis. Furthermore, many pyrimidine-5-carbonitrile derivatives are proved to exhibit potent anticancer as well as antimicrobial activities. Patients with neoplastic disorders are mostly subjected to microbial infections. The co-administration of multiple drugs for treating patients suffering from cancer disease accompanied with microbial infections, which may inflect some added health problems, especially in patients with impaired liver and/or kidney functions. Therefore, the concept of monotherapy by a single drug which possesses dual utility may be advantageous from both therapeutic as well as cost-effective stand points. Consequently, our efforts are devoted to synthesize and investigate innovative pyrimidine analogs with dual function; anticancer/

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antimicrobial. Aligned with this scope, we synthesized pyrimidine Schiff bases bearing biologically active functionalities and studied their biological activity in detail, which we report in this paper.

2. Experimental

2.1. Materials and methods

Melting points were determined using open glass capillaries on a Raaga melting point apparatus. Microanalytical data of the compounds were recorded in the ElementarVario EL IIICHN analyzer. The FT-IR spectra of the samples were recorded on a Shimadzu spectrophotometer in the wavenumber range of 4000–400 cm^{-1}. ^1H NMR spectra were recorded on a Jeol 400 MHz spectrometer using TMS as an internal standard. Absorption spectral measurements were performed using a double beam UV–visible spectrophotometer (Jasco V-630). A spectrofluorimeter (PerkinElmer LS55), equipped with a 120 W xenon lamp for excitation, was used for the measurement of fluorescence. Both the excitation and the emission bandwidths were set up at 4 nm. The antimicrobial activities of the Schiff bases were carried out by well diffusion method. The anticancer activity of the compounds was carried out against the breast cancer cell line MCF-7 using MTT assay. The antitubercular activity was carried out using microplate alamar blue assay.

2.2. Synthesis of the Schiff bases

A mixture of 5,6-diamino-2-mercapto-5,6-dihydro-1*H*-pyrimidin-4-one (0.15 g, 1 mmol) and salicylaldehyde (0.12 mL, 1 mmol) or 5-bromo salicylaldehyde (0.20 g, 1 mmol) in 95% ethanol was refluxed for 2 h. The precipitate was filtered and washed with ethanol.

2.2.1. 6-Amino-5-[(2-hydroxybenzylidene)amino]-2-thioxotetrahydropyrimidin-4-one***(1)***

Crystallized from DMF/H_2O. Yield 78%. M.p.: 237 °C. Calculated for $C_{14}H_{17}N_5O_3S$ (334.38): C, 49.84; H, 5.68; N, 20.76. Found C, 49.79; H, 5.63; N, 20.70. IR (cm^{-1}): 1554 (C═N), 858 (C═S). UV–Vis (nm): 257, 310. ^1H NMR (ppm): 6.76–8.15 (m, 4H, aromatic), 7.86 (s, 1H, -CH═N), 8.11 (s, 2H, NH), 11.57 (s, 2H, -NH_2), 4.54 (s, 1H, -OH), 13.18 (s, 1H, -CHO), 3.48 (s, 6H, -CH_3).

2.2.2. 6-Amino-5-[(2-bromo-6-hydroxybenzylidene)amino]-2-thioxotetrahydropyrimidin-4-one***(2)***

Crystallized from DMF/H_2O. Yield 75%; M. p.: 254 °C. Calculated for $C_{14}H_{16}BrN_5O_3S$ (415.03): C, 40.39; H, 4.36; N, 16.82. Found C, 40.31; H, 4.29; N, 16.76. IR (cm^{-1}): 1560 (C═N), 887 (C═S). UV–Vis (nm): 257, 288. ^1H NMR (ppm): 6.85–8.45 (m, 3H, aromatic), 7.93 (s, 1H, -CH═N), 8.52 (s, 2H, NH), 11.71 (s, 2H, -NH_2), 4.54 (s, 1H, -OH), 13.18 (s, 1H, -CHO), 3.48 (s, 6H, -CH_3).

2.3. Determination of X-ray crystal structure

A Bruker APEX-2 X-ray (three-circle) diffractometer was employed for crystal screening, unit cell determination and data collection. The X-ray radiation employed was generated from a Mo sealed X-ray tube (Kα = 0.7017 Å with a potential of 40 kV and a current of 40 mA) fitted with a graphite monochromator in the parallel mode (175 mm collimator with 0.5 mm pinholes). Systematic reflection conditions and statistical tests of the data suggested the space group P121/c1. A solution was obtained readily using SHELXTL (XS). Hydrogen atoms were placed in idealized positions and were set riding on the respective parent atoms. All non-hydrogen atoms were refined with anisotropic thermal parameters [19]. The structure was refined (weighted least squares refinement on F^2) to convergence. Olex-2 was employed for the final data presentation and structure plots [20]. Supplementary material for the crystal structure has been deposited with the Cambridge Crystallographic Data Centre (CCDC No.: 1,012,277 **(1)** and 1,048,130 **(2)**; deposit@ccdc.cam.ac.uk or http://ccdc.cam.ac.uk).

2.4. In vitro antimicrobial activities

The antimicrobial activities of the ligand and complexes were tested against the species *Pseudomonas aeroginosa*, *Escherchia coli*, *Thiobacillus thidurance*, *Serratia marcescens*, *Acinetobater baumauii*, *Aspergillus niger* and *Candida albicans* by well diffusion method using agar nutrient medium. The test organisms were grown on nutrient agar for antibacterial and potato dextrose for antifungal studies in Petri plates. The plates were incubated for 24 and 72 h for bacteria and fungi respectively. Then, the test solutions were diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration of the samples was noted. DMF was used as the negative control, ciprofloxacin was used as positive standard for antibacterial and fluconazole for antifungal studies. The minimum inhibitory concentration was determined by serial dilution method.

2.5. In vitro anticancer activity:Cell treatment procedure and MTT assay

The monolayer of cells was detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/mL. Cell suspensions were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37 °C. After 24 h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in DMSO and diluted twice to reach the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 μL of these different sample dilutions were added to the appropriate wells already containing 100 μL of the medium, resulting in the required final sample concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 °C. The medium without samples served as control and triplicate was maintained for all concentrations.

3-[4,5-Dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15 μL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 μL of DMSO and then measured the absorbance at 570 nm using a microplate reader. The percentage cell inhibition was determined using the formula, % cell inhibition = [100 − Abs (sample)/Abs (control)] × 100. Nonlinear regression graph was plotted between % cell inhibition and $\log_{10}$ (concentration) and IC_{50} was determined using graph pad prism software [21].

2.6. DNA binding studies

A solution of CT-DNA in 50 mM NaCl/5 mM Tris-HCl (pH 7.0)