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Original article

Molecular docking study, synthesis and biological evaluation of Schiff bases as Hsp90 inhibitors

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ABSTRACT

Heat shock protein 90 (Hsp90) is an emerging attractive target for the discovery of novel cancer therapeutic agents. Docking methods are powerful *in silico* tools for lead generation and optimization. In our mission to rationally develop novel effective small molecules against Hsp90, we predicted the potency of our designed compounds by Sybyl surflex Geom X docking method. The results of the above studies revealed that Schiff bases derived from 2,4-dihydroxy benzaldehyde/5-chloro-2,4-dihydroxy benzaldehyde demonstrated effective binding with the protein. Subsequently, a few of them were synthesized (1–10) and characterized by IR, ^{1}HNMR and mass spectral analysis. The synthesized molecules were evaluated for their potential to suppress Hsp90 ATPase activity by Malachite green assay. The anticancer studies were performed by 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay method. The software generated results was in satisfactory agreement with the evaluated biological activity.

1. Introduction

Heat shock protein 90 (Hsp90) is a type of molecular chaperone that plays a crucial role in the repair of diverse range of "client" proteins (defined as proteins whose level declines upon treatment with Hsp90 inhibitor) involved in the development and progression of malignancy [1,2]. Therefore, compounds with an ability to attenuate Hsp90 function is considered to be broad spectrum in activity with reduced liability for acquired drug resistance [3]. The function of Hsp90 is driven by hydrolysis of ATP to ADP in the N-terminal domain of the protein followed by ATP/ADP exchange. Blocking of this ATPase activity will lead to cessation of Hsp90 function [4,5]. Hence, majority of Hsp90 inhibitors developed so far competitively dock to the N-terminal ATP binding pocket of the protein [6].

The resorcinol moiety was found to be involved in prominent hydrogen bonding and hydrophobic interactions with the amino acid side chain belonging to the N-terminal ATP binding cleft of Hsp90 [7–9]. This was reflected in the SAR study of various compounds tested for Hsp90 suppression potential wherein analogues without the two hydroxyl groups were much less potent in the binding assay [10–12]. Moreover, the resorcinol scaffold is suggested to be devoid of any toxic side effects commonly associated with other fragments like quinone, halopyrimidine, etc. [13]. Literature review on anticancer agents revealed that Schiff bases (imines or azomethines) possess cytotoxic properties with no concrete evidence of their mechanism of destroying the cancer cells [14–22]. Studies further indicated that the aldehyde substituent of azomethines were superior to the amine portion in exhibiting cytotoxic effects [23]. Furthermore more recently, two imines used as a precursor for azetdine ring synthesis have been reported to moderately diminish Hsp90 activity [24]. In view of the fact that dihydroxy phenyl group is an established template for Hsp90 inhibition and considering the significant anticancer activity displayed by imines, we performed docking studies of a series of Schiff bases of 2,4-dihydroxy benzaldehyde and 5-chloro-2,4-dihydroxy benzaldehyde derivatives (Fig. 1). The ligands, which fared well in the docking program were synthesized and characterized by IR, ^{1}HNMR and mass spectroscopic studies. A colorimetric assay (malachite green assay) was used to measure the extent of Hsp90 ATPase activity inhibition by our synthesized compounds. This assay determines the amount of free phosphate liberated due to

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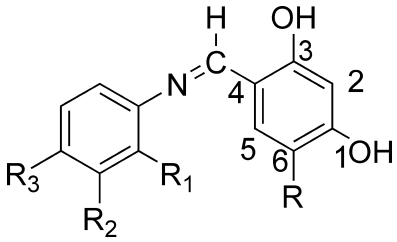

Fig. 1. General structure of Schiff bases synthesized with numbering system used in this report.

ATP hydrolysis and is based on the reaction of phosphomolybdate complex with malachite green. The synthesized molecules were further screened for cytotoxic activity on PC3 prostate cancer cells. The results of the above studies are presented in this paper.

2. Materials and methods

2.1. Molecular docking studies

The docking studies was carried out with Surflex Geom X programme of Sybyl X-1.2 version softwares installed on Dell Precision T-1500 workstation [Intel(R) Core(TM) i7 CPU 860 @ 2.80 GHz 2.79 GHz; 12.0 GB RAM, 1 TB Hard disk]. Crystal structure of Hsp90 was selected from the Protein Data Bank (PDB ID: 3EKR) with a resolution of 2Å [25]. The protein preparation step involved addition of hydrogen and removal of water molecules except 902, 903, 981 and 1026 [26,27]. These four water molecules were revealed to be important for effective ligand-protein binding [27–31]. The protein was energy minimized using conjugate minimization technique of Powell [32]. The protomol (idealized active site) was generated from hydrogen-containing protein mol2 file by keeping the default parameters (threshold factor of 0.5Å and a bloat of 0Å) [33,34]. The ligands were first drawn in Chem draw, saved as mol files and then converted into SD file format by using Schrodinger software (Maestro, 9.1 versions). The ligands were then prepared for docking by first generating a clean 3D conformation using Concord program which generates 20 conformations per structure [32,35]. This is followed by filtering structures based on drug-likeness and docking them with the prepared protein at the developed protomol [36].

2.2. Synthesis of compounds

The chemicals, reagents and solvents employed for synthesis were procured from Hi-media Laboratories Private Limited, Merck specialties Private Limited and SD fine-chem limited. The progress of the reaction and purity was monitored by using TLC Silica gel 60 F_{254} aluminium sheets (Merck F_{254}, Darmstadt, Germany) developed in mobile phase containing ethyl acetate and petroleum ether (1:1). The melting point of the synthesized compounds was determined by DRK Digital melting point apparatus. IR spectra were recorded on Shimadzu IR-Affinity spectrometer using KBr pellets. The ^{1}HNMR spectra of the compounds synthesized were acquired in deuterated DMSO on a Bruker ARX 400/300 MHz (Bruker AG, Fallanden, Switzerland) instrument. Tetramethylsilane was used as the internal standard and all chemical shift values were expressed in parts per million (δ, ppm). The mass spectra were obtained from 6120 Quadrupole LC/MS mass spectrometer using atmospheric pressure-electron spray ionization method (Agilent Technologies, California, USA).

2.2.1. Synthesis of 2,4-dihydroxy benzaldehyde [37]

This was prepared according to a reported procedure (Fig. 2). A two necked round bottom flask was charged with DMF (2.37 mL, 0.0306 mole) and acetonitrile (7 mL). This was followed by addition of $POCl_3$ (2.43 mL, 0.026 mole) drop wise to the reaction mixture by maintaining the temperature between 22 °C to 28 °C. The reaction was stirred at 22–28 °C for 1 h. The solution remains clear throughout. Subsequently the reaction mixture was cooled in a dry-ice bath to −15 to −17 °C and a solution of resorcinol (2.5 g, 0.022 mole) in acetonitrile (7 mL) was slowly added. Precipitation of the Vilsmeier formamidinium phosporodichloridate salt occurs during this addition. The reaction was stirred for an additional 2 h at −15 to −17 °C and then at 28–32 °C for 1 h. The reaction was cooled to 5 °C and after stirring for 1 h the product is isolated by filtration and washed with cold acetonitrile. The intermediate salt was added portion wise to a beaker containing water (62 mL) stirred at 40 °C. The reaction was heated to 52 °C for 0.5 h, and then cooled. When the temperature has reached 35 °C sodium thiosulfate solution (0.09 M, 1 mL) was added to discharge the resulting pink coloration. The reaction was cooled to 5 °C and stirred for 2 h. The mixture was then filtered; the white solid was washed with cold water and air dried for 2 h to obtain a white crystalline solid.

2.2.2. Synthesis of 5-chloro-2,4-dihydroxy benzaldehyde [38]

2, 4-dihydorxy benzaldehyde (3.5 g, 0.0253 mole) was dissolved in 100 mL of water and 24 mL of 4% sodium hydroxide contained in a beaker. This solution is cooled to 20 °C and mixed with 200 mL of sodium hypochlorite solution. The temperature was maintained at 20 °C for 1 hour with occasional stirring. Upon acidifying with HCl a light yellow precipitate of the product was obtained. It was recrystallized from methanol/water to obtain pure crystals of 5-chloro-2,4-dihydroxy benzaldehyde (Fig. 2).

2.2.3. Synthesis of 2,4-Dihydroxy benzaldehyde/5-chloro-2,4-dihydroxy benzaldehyde derived Schiff base derivatives

2,4-dihydroxy benzaldehyde (0.5 g, 0.0036 mole)/5-chloro-2,4-dihydroxy benzaldehyde (0.5 g, .0028 mole) and eqimolar concentration of various aniline derivatives was transferred to a round bottom flask containing absolute ethanol sufficient enough to dissolve the added reagents. The reaction mixture was then refluxed for 3 h (Fig. 2). The TLC was monitored until completion of the reaction. Subsequently water was added resulting in the formation of colored solid, which was filtered and air dried. It was further recrystallized from methanol to obtain the pure product. The general structure of the ligands with the numbering system used in this work is shown on Fig. 1 with detail of the exact structures given in Table 1.

2.3. HSP 90 ATPase inhibitory activity

The plasmid pRSETA encoded His_6-tagged human Hsp90β (hHsp90β) was a kind gift from Dr. Chrisostomos Prodromou, University of Sussex, United Kingdom. Expression and purification of the chaperone was achieved as described earlier [5]. ATPase activity was measured following a modification of previously reported procedure [39,40]. Briefly, 10 μg of pure hHsp90β was incubated with the inhibitor to be tested (geldanamycin was used as a standard drug) for 10 min at 20 °C in a buffer containing 50 mM Hepes at pH 7.5, 6 mM $MgCl_2$, 20 mM KCl, and 1 mM ATP. The reaction was stopped by the addition of two volumes of malachite green reactive solution prepared as described by Harder et al. [41]. After 25 min at room temperature, the absorbance at 630 nm was measured [42,43].

2.4. In vitro cell viability assay

The compounds were screened for their cytotoxicity on PC3 prostate cancer cells by adopting the MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] based cell proliferation assay method [44,45]. The carcinoma cell lines were harvested