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

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ABSTRACT

The ubiquitously expressed heat shock protein 90 is an encouraging target for the development of novel anticancer agents. In a program directed towards uncovering novel chemical scaffolds against Hsp90, we performed molecular docking studies using Tripos-Sybyl drug designing software by including the required conserved water molecules. The results of the docking studies predicted Mannich bases derived from 2,4-dihydroxy acetophenone/5-chloro 2,4-dihydroxy acetophenone as potential Hsp90 inhibitors. Subsequently, a few of them were synthesized **(1–6)** and characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. The synthesized Mannich compounds were evaluated for their potential to suppress Hsp90 ATPase activity by the colorimetric Malachite green assay. Subsequently, the molecules were screened for their antiproilferative effect against PC3 pancreatic carcinoma cells by adopting the 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay method. The activity profile of the identified derivatives correlated well with their docking results.

1. Introduction

The 90 kDa, heat shock protein (Hsp90) is an important member of a class of proteins known as molecular chaperones [1]. These chaperones play a critical role in maintaining the structure and function of other "client" polypeptides within a cell. Among all the chaperone proteins, Hsp90 has emerged as an attractive target for cancer chemotherapy because of its involvement in the repair of multiple oncogenic proteins [2,3]. Furthermore this protein is over expressed in carcinoma cells in association with co-chaperones whereas Hsp90 of normal cells resides in a free uncomplexed form [4,5]. Moreover several proteins responsible for the development of resistance to antineoplastic agents are also customers of Hsp90. Hence inhibitors of this chaperone are considered to be broad spectrum in activity with fewer toxic side effects and reduced liability for acquired drug resistance [6,7]. The healing function of Hsp90 is guided by hydrolysis of ATP to ADP within the N-terminal domain of the protein. Blocking of this ATPase activity leads to attenuation of Hsp90 function [8]. Hence, majority of Hsp90 inhibitors under preclinical and clinical investigation competitively dock to this cleft of the protein [9,10]. The druggability of Hsp90 chaperone was first established in 1994 using the natural product geldanamycin [11]. Since then 17 agents have entered various phases of clinical trials. However till date none of them were approved for human use by any regulatory agencies [12]. Hence rational design of novel class of compounds against Hsp90 is the need of the hour. Furthermore an enormous amount of time and capital is involved in bringing a new molecule to the market. Hence cost effective discovery of small molecular weight Hsp90 inhibitors remains an active field of research and has been reviewed extensively.

The dihydroxy phenyl scaffold was found to be involved in prominent hydrogen bonding and hydrophobic interactions with various amino acid residues at the N-terminal ATP binding cleft of Hsp90 [13–16]. Furthermore, the study of structure activity relationship for Hsp90 inhibitors indicated that removing any one of the hydroxyl group leads to molecules with much lesser binding efficiency [17,18]. Moreover, the resorcinol moiety is suggested to

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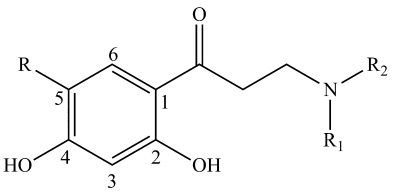

Fig. 1. General structure of Mannich bases synthesized with numbering system used in this work.

be devoid of any toxic adverse effects commonly associated with other fragments like quinone, halopyrimidine, diiodo phenyl, etc. [19]. Literature survey on anticancer agents revealed that Mannich bases possess antiproliferative properties with no concrete evidence of their mechanism of killing the cancer cells [20–28].

Structure based drug design is an efficient approach that predicts the activity of molecules within a short span of time [29]. In view of the fact that dihydroxy phenyl group is an established template for Hsp90 antagonism and considering the importance of Mannich bases in cancer chemotherapy, we performed structure guided drug designing via molecular docking studies for a series of 2,4-dihydroxy acetophenone and 5-chloro-2,4-dihydroxy acetophenone derived Mannich bases (Fig. 1). The ligands (**1–6**) which fared well in the docking program were synthesized and characterized by IR, ^{1}H NMR, ^{13}C and mass spectroscopic studies. The malachite green colorimetric assay was used to measure the extent of Hsp90 ATPase activity attenuation by our synthesized compounds. This assay accurately determines the amount of free inorganic phosphate liberated due to ATP hydrolysis and is based on the reaction of phosphomolybdate complex with malachite green. The synthesized dihydroxy phenyl propanone analogues were further screened for their antiproilferative effect on PC3 prostate cancer cell lines by utilizing the well-established 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. The results of the present studies open the possibility to expand the molecular diversity space of Hsp90 inhibitors.

2. Materials and methods

2.1. Structure based drug design

The receptor based drug designing process was achieved with Surflex Geom X docking tool of Sybyl X-1.2 version software installed on a Dell Precision T-1500 workstation. A co-crystal structure of 4-{[(2R)-2-(2-methylphenyl)pyrrolidin-1-yl]carbonyl}benzene-1,3-diol and human Hsp90 submitted to protein data bank (PDB ID: 3EKR, resolution = 2 Å) was utilized for the docking procedure [30]. The four conserved waters (902, 903, 981 and 1026) were retained during protein preparation as they provide key interactions in stabilizing the ligand at the active site [31,32]. This was followed by addition of hydrogens for the amino acid residues and waters. The AMBER7 FF99 force field was utilized for the purpose of energy minimization which was followed by protomol generation [33]. A protomol is an idealized representation of a ligand that makes every potential interaction with protein's binding site. The protomol was created by extracting the original ligand (PDB ID: 3EKR) with the help of MOLCADD program of the software. A threshold [Threshold is a factor (from 0 to 1) determining how much the protomol can be buried in the protein. Increasing this number decreases the volume] of 0.5 and a bloat (bloat is used to inflate the protomol and include the nearby crevices) of 0 was maintained during the process of protomol generation [13]. The Mannich bases were first drawn in Chem draw ultra 8.0 and exported as mol files to Maestro, 9.1 versions of Schrodinger software for converting into Sybyl compatible SD file format. Subsequently, a clean, energy minimized, 3D conformation of the ligands were generated using Concord program (random perturbation of the position and conformation of the supplied ligand followed by bump relaxation) and filtered based on drug-likeness. Finally, 50 recacetophenone/5-chloro resacetophenone derived Mannich compounds were docked at the virtual active site via Geom X method by considering 20 poses per ligand [34–36]. The binding affinity of the ligands is predicted by the software in terms of total score which is expressed as—log K_d, where K_d is binding constant. A high value of total score indicates good protein–ligand binding.

2.2. Synthesis of compounds

All the chemicals and organic solvents used in the synthesis of compounds were purchased from Hi-media Laboratories Private Limited, Merck specialties Private Limited and SD fine-chem limited. The reactions were monitored by analytical thin layer chromatographic analysis (stationary phase: florescent F_{254} containing silica gel 60 G coated on aluminium sheets; mobile phase: 10% methanol in DCM). Uncorrected melting point values were determined by using a DRK Digital melting point apparatus. Infra red (IR) spectra were recorded in powder form on Shimadzu IR-Affinity spectrophotometer using Shimadzu diffuse reflectance attachment (DRS-800). ^{1}H NMR (300/400 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Bruker Avance instrument. Chemical shift values are reported in parts per million (δ, ppm) downfield from tetramethyl silane (TMS) as an internal reference standard and coupling constants (J) are given in Hz. Mass spectra were run using a atmospheric pressure-electron spray ionization 6120 Quadrupole LC/MS mass spectrometer (Agilent Technologies, California, USA).

2.2.1. Preparation of 5-chloro resacetophenone [37]

12.7 g (0.0040 mol) of freshly fused anhydrous zinc chloride is dissolved with the aid of heat in 6 ml of glacial acetic acid in a beaker. This was followed by addition of 5 g (0.0340 mol) of 4-chloro resorcinol to the hot solution with constant stirring. The reaction mixture is then heated on a sand bath until it just begins to boil (about 120 °C). The flame is then removed and the reaction is allowed to complete itself at a temperature not exceeding 140 °C. The reaction mixture was allowed to stand on the sand bath for 20 min without heating. This was followed by addition of 25 ml of 0.1N hydrochloric acid to the reaction mass. The resulting dark red solution upon cooling to 5 °C resulted in the formation of a precipitate which was filtered and washed with dilute hydrochloric acid. It was further purified by column chromatography (ethyl acetate/petroleum ether solvent system as the eluent and 60–120 mesh size silica gel as the stationary phase) to obtain light brown color product (Fig. 2).

Resacetophenone was procured from commercial vendors.

2.2.2. General procedure for the synthesis of dihydroxy phenyl propanone Mannich base [38]

A mixture of the resacetophenone/5-chloro resacetophenone (0.0054 mol), paraformaldehyde (0.0059 mol) and appropriate secondary amine salts (0.0054 mol) was transferred to a round bottom flask. To it 30 ml of 95% ethanol was added followed by addition of 1–2 drops of concentrated hydrochloric acid. The reaction mixture was then refluxed for 3 h. The completion of the reaction was ascertained by ascending thin layer chromatographic technique. Thereafter the solvent was removed under vaccum to obtain the product (Fig. 2). The Mannich bases were further purified by using column chromatographic method over 100–200 mesh silica gel using methanol/dichlromethane solvent system as the eluent. The general structure of the ligands along with their numbering